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On the Division of the Genus *Oesophagostomum* Molin, 1861, into Subgenera and the Creation of a New Genus for the Oesophagostomes of the Wart-Hog.

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During the past nine years the writer has collected many helminths from the gastro-intestinal tract of various antelopes in Northern Rhodesia.

Amongst the collected material are oesophagostomes from the Puku (*Adenota vardoni*), the Red Lechwe (*Onotragus leche*), the Duiker (*Cephalophus natalensis*), the Kudu (*Strepsiceros strepsiceros*) and the Roan Antelope (*Hippotragus equinus*).

The character of the internal leaf-crown observed in the species from the Puku and the Red Lechwe induced the examination of this structure in all the different species available at the London School of Hygiene and Tropical Medicine. It was observed that the number of elements in the two leaf-crowns of a species were identical in the oesophagostomes of ruminants. Goodey (1924) records the buccal capsule in *O. asperum* to be composed of a ring of twelve concavo-convex elements placed side by side, the top of each carrying an element of external leaf-crown and two scales of the internal leaf-crown. He observes that the inner face of each portion of buccal capsule carries a transparent layer of cuticle grooved horizontally about two thirds of its length from the anterior end. This horizontal groove observed by Goodey corresponds to the point of origin of the internal leaf-crown elements and the longitudinal lines between the so-called concavo-convex elements of the buccal capsule are merely the lateral limits of the external elements. The buccal capsule and internal leaf-crown seem to be merely the base of the external elements having become chitinised. The so-called internal elements in *O. asperum* are long concavo-convex bits of chitinised material, lying close together, and it is their length and their curvature which accounts for the " scales "

to appear short and in pairs as figured by Goodey. In reality a pair is composed of the lateral borders of two adjoining elements and this accounts for the two minute scales to show at the base of each external element.

Goodey in his description of *O. venulosum* refers to Railliet's (1913) statement that there were 18 elements in the external leaf-crown and 18 scales in the internal leaf-crown and observes that Railliet's figure gives the impression of each element being made up of two rods joining above to form a common point. Railliet evidently took the adjoining lateral margins of adjoining elements as one " scale." Ransom (1911) records for *O. venulosum* " . . . ; two crowns of leaf-like processes ; not more than 16 processes in each crown." He does not figure these elements.

Thornton (1924) remarks on Ransom's description and states :— " There is no doubt that, as in *O. columbianum*, the external leaf-crown is composed of twice as many elements as the internal." Canavan (1931) describes *O. vigintimembrum* from a camel (*Camelus dromedarius*) and in a rather brief description of the species points out : " The cervical papillae are on a level with the oesophageal connection to the intestine." He records the external leaf-crown to be composed of 20 elements with 20 bipartite elements in the internal crown. He illustrates (Pl. IX., Fig. 12) the external and internal leaf-crowns. What he figures as the external leaf element is undoubtedly only a portion of that element. He records the width and the depth of the buccal capsule as 0·074 mm. and 0·019 mm. respectively. The spicules are described : " Equal, tips fused." The tips of the alated spicules often appear fused. In the oesophagostomes the spicules are not fused.

Vuylsteke (1935) describes *O. rodhaini* from *Okapi Johnstoni* but his description and the accompanying figures of the buccal capsule and the leaf crowns are somewhat unsatisfactory. His Fig. A.1 suggests 28 rather long elements in the internal leaf-crown while Fig. A.2 shows the wall of the buccal capsule diverging anteriorly with the internal leaf-crown arising from the anterior border of the capsule.

Prof. Leiper very kindly loaned me the two males, recorded as *Oesophagostomum okapi* Leiper, 1935, for examination. The buccal capsule and the leaf-crowns are somewhat like those of the oesophagostome recovered from the Puku and the Red Lechwe. The absence of well

developed lateral alae and the position of the cervical papillae serve to differentiate between the two species. The cervical papillae in *O. okapi* were on a level with the maximum diameter of the oesophagus and not so posteriorly placed as in the species described by Vuylsteke and Canavan. The height and the width of the buccal cavity anteriorly were practically the same as given for *O. vigintimembrum*. The number of elements in each leaf-crown appears to be twenty. The external elements are well developed, long and pointed anteriorly and not short with rather rounded anterior extremities as figured for those of *O. vigintimembrum*. In the *O. okapi* the anterior extremity of the internal elements is directed laterally and not medially as in *Oesophagostomum* (*Pukuia*) *lechwei* sp. n. In *O. okapi* the cephalic inflation is of the type described for the members of the subgenus *Hysteracrum* Railliet and Henry, 1913.

Canavan (1931) records *O. venulosum* from the same camel and reports sixteen rounded external leaf-crown elements and sixteen internal elements with twice as many processes. An examination of *O. venulosum* proved the internal elements to appear " bipartite " as recorded for *O. vigintimembrum* but this " bipartite " appearance was due to the elements being concavo-convex. The same was also observed in *O. columbianum*. Goodey (1924) reports the inner leaf-crown of *O. columbianum* to be composed of " 40–48 rather long and narrow elements, two to each element of external leaf-crown." The elements of Goodey are merely the lateral borders, inwardly directed, of the elements. In the species *O. dentatum* the internal leaf-crown elements arise from about the middle of the buccal wall and are short and straight but the lateral borders are directed latero-medially causing the inner surface to be grooved. The " notching " of the anterior extremity was therefore not so marked as in the other species mentioned above. In a face-on position the lateral borders of the teeth, projecting into the buccal capsule, create the impression of two separate elements being present. The corresponding elements in the case of *O. quadrispinulatum* are more anteriorly placed on the wall of the buccal capsule and are directed almost horizontally into the buccal cavity with the result that the notching is still less evident than in *O. dentatum*. Travassos and Vogelsang's (1932) illustrations of *O. dentatum* are undoubtedly those of *O. quadrispinulatum*. Their Figs. 2, 4 and 5 show four internal elements to each external element. In the illustrations of *Oes.* (*Conow.*) *blanchardi* four internal elements are likewise figured to each external leaf element.

In the *Oes.* (*Ihleia*) *stephanostomum* the external leaf elements are poorly developed and not unlike the so-called internal leaf elements in *Oes.* (*Bosc.*) *radiatum.* This suggests that the so-called internal leaf-crown in *Oes.* (*Bosc.*) *radiatum* may in reality be the external leaf-crown.

Sub-division of the Genus *Oesophagostomum* Molin, 1861.

Railliet and Henry (1913) created the subgenus *Oesophagostomum* (*Hysteracrum*) for the species *O. venulosum* and *O. asperum* in which the cervical papillae are situated posterior to the termination of the oesophagus and the subgenus *Oesophagostomum* (*Proteracrum*) for the species *O. columbianum* and *O. radiatum* which possess little or non-inflated cephalic vesicles ; are furnished with well developed lateral alae and whose third and fourth stage larvae produce parasitic nodules in the intestinal wall of their hosts.

Ihle (1922) redescribes *O. apiostomum* and finding that it differed in certain essential characters from the other species created the new subgenus (*Conoweberia*) for *O. apiostomum* and *O. brumpti* in which the oesophagus is furnished with a well defined oesophageal funnel armed with three curved teeth.

Daubney (1924) states that *O. mwanzae* was most closely related to the members of the subgenus *Conoweberia* but admits two years later that it was more closely related to *O. dentatum* as described by Goodey (1924).

Goodey (1924) in his redescription of the four species of oesphagostomes from domesticated stock, criticises the subdivision proposed by the French workers and expresses the view that the genus should be maintained

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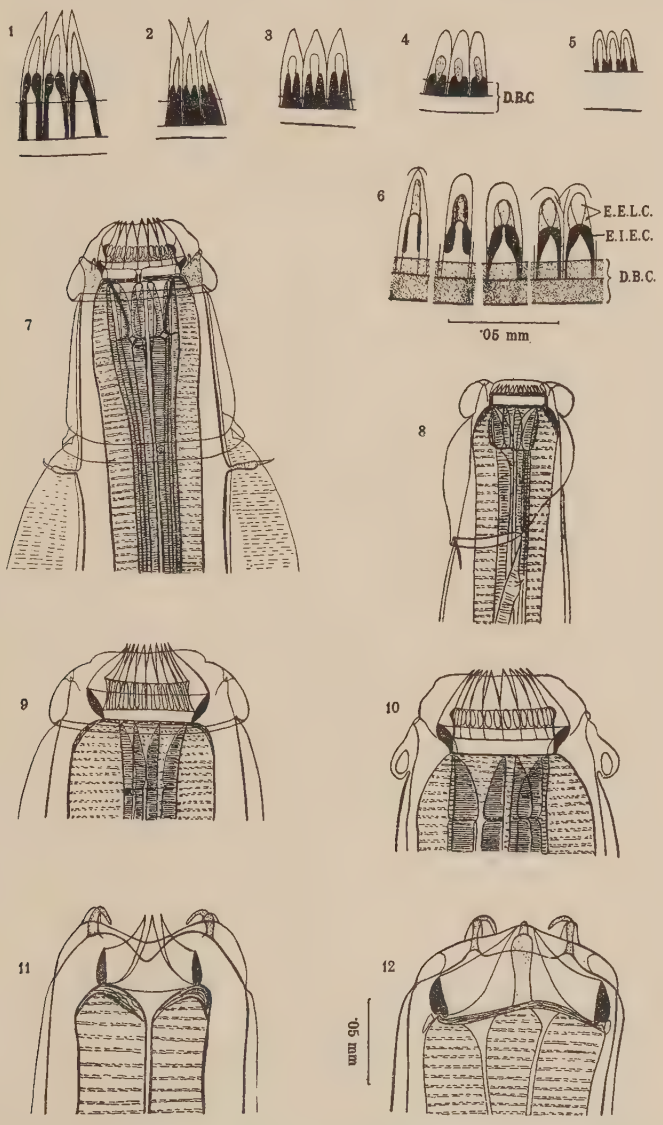
Fig. 1.—External and internal leaf-crown elements of *Oes.* (*Pukuia*) *lechwei.*
Fig. 2.—External and internal leaf-crown elements of *Oes.* (? *Hyst.*) *okapi.*
Fig. 3.—External and internal leaf-crown elements of *Oes.* (*Prot.*) *columbianum.*
Fig. 4.—External and internal leaf-crown elements of *Oes.* (*Hyst.*) *venulosum.*
Fig. 5.—External and internal leaf-crown elements of *Oes.* (*Hud.*) *multifoliatum.*
Fig. 6.—Lateral view of external and internal leaf-crown elements at different levels : *Oes.* (*Puk.*) *lechwei.*
Fig. 7.—Ventral view of anterior end : *Oes.* (*Puk.*) *lechwei.*
Fig. 8.—Lateral view anterior end : *Oes.* (*Hud.*) *roscoei.*
Fig. 9.—Anterior end of *Oes.* (*Hysteracrum*) *okapi.*
Fig. 10.—Anterior end of *Oes.* (*Puk.*) *lechwei.*
Fig. 11.—Lateral view of anterior end : *Daubneyia oldi.*
Fig. 12.—Dorsal view of anterior end : *Daubneyia oldi.*

intact. Thornton (1924) and Daubney (1926) agree with Goodey that the genus should not be sub-divided. Baylis and Daubney (1926) and Yorke



and Maplestone (1926) disregard the sub-divisions created by the two French helminthologists. Sandground (1929) described a new parasite

(*Bosicola tricolleris*) from cattle in Mexico. This species proved to be *Oes.* (*Proteracrum*) *radiatum*.

Travassos and Vogelsang (1932) revived the four already proposed subgenera and, because the species from cattle and sheep differed in several characters, they proposed *Bosicola* as an additional subgenus with *Oes.* (*Bosicola*) *radiatum* as type and included *O. curvatum* Maplestone, 1931.

Baylis (1936) disagrees with Travassos and Vogelsang but accepts *Bosicola* as a genus for the species *O. radiatum*, *O. curvatum* and *Bourgelatoides traguli* Chandler, 1931.

Travassos and Vogelsang also created the additional subgenus *Ihleia* for the species *O. stephanostomum* Stossich, 1904 and *O. ventri* Thornton, 1924 but name the latter *Oesophagostomum* (*Conoweberia*) *ventri* Thornton, 1924.

A subdivision of the genus into closely related groups seems quite in order and warranted. This division will be better appreciated when once the development of the third and fourth stage larval forms of the different species in their respective hosts have been ascertained.

The new subgenus *Hudsonia* is proposed for the reception of the species *Oesophagostomum multifoliatum* Daubney and Hudson, 1932, from sheep and goats in Kenya Colony, *Oesophagostomum walkeri* Mönnig, 1932 from the Eland in Kenya Colony and *Oesophagostomum africana* Mönnig, 1932, from the Springbuck in South Africa.

Oesophagostomum (*Hudsonia*) *multifoliatum* Daubney and Hudson, 1932 is designated type of the new subgenus. Specimens of this species are present in collections of oesophagostomes from cattle in Tanganyika Territory and Southern Rhodesia in the helminthological collection at the London School of Hygiene and Tropical Medicine.

The various measurements given by Mönnig for *O. africana* are almost identical with those given for *O. multifoliatum* by Daubney and Hudson. Neither of these authors states the height of the buccal capsule of the species concerned. At the British Museum (Natural History), I had the opportunity of examining three males of *O. multifoliatum* from cattle in Kenya Colony and found the height of the buccal capsule to be approximately 0·016 to 0·02 mm., while the drawings by Daubney and Hudson suggest a much greater depth. The worms might not have been specimens of *O. multifoliatum* but they were identical with the worms from the same

host in the other two countries mentioned above. The wall of the buccal cavity converges a little in its posterior third, but finally tends to diverge as described for the species from sheep and goats. The length of the spicules in the specimens from cattle varied very little in the five specimens examined by me. It was 0·6 to 0·65 mm. Daubney and Hudson give the measurements as 0·75 to 0·85 mm. In *O. africana* they vary from 0·75 to 0·84 mm. The measurements of the ova, *in utero*, were on an average 0·135 mm. by 0·075. The only real differences between the species from cattle and the species described from sheep and goats seem to be the depth of the buccal capsule as figured by Daubney and Hudson and the degree of development of the cephalic inflation on the dorsal aspect. A single female oesophagostome from Thomson's Gazelle (*Gazella Thomsoni*) from Tanganyika Territory proved to be related to the species of the subgenus *Hudsonia*. It is according to the measurements of the ova and of the buccal capsule closely related to the specimens from the Kudu (*Strepsiceros strepsiceros*), Duiker (*Cephalophus natalensis*) and Eland (*Taurotragus orynx*). The cephalic inflation was not as well developed as in the specimens from these hosts. It appeared with some wrinkles suggesting a fair amount of shrinkage. The lateral alae are well developed and show up prominently on the level with the vulva where they attain a height of 0·105 mm. while the width of the body not including the alae was 0·3 mm. Neither Daubney and Hudson (1931) nor Mönnig (1931) remark on this marked development of the alae in the region of the vulva in the species described by them.

In the description of the oesophagostomes more attention should be given to the various measurements and other characters of the buccal capsule than has been the case hitherto. The size of the most matured ova in the uterus and the ratio of the height of the buccal capsule to its internal diameter would seem to be of some significance in differentiating between closely related species.

When collecting oesophagostomes the intestinal wall should be examined for larval cysts, caseous and calcified parasitic nodules. In some of the species parasitizing man, monkeys, apes and rodents the worms develop to almost full size in submucosal cysts. Some of the species from domesticated ruminants cause hardly any nodules in the intestinal wall. The specimens from cattle showed a slight inflation but this was probably due to the methods of collection and preservation. Variations in degree of inflation

are often very marked in specimens of *Oes.* (*Bosicola*) *radiatum*, *Oes.* (*Oes.*) *dentatum* and others although collected from the same host and treated similarly during the process of preservation.

The existing descriptions of *Oes.* (*Hudsonia*) *multifoliatum* and *Oes.* (*Hudsonia*) *africana* when compared with the measurements of the specimens from cattle seem to suggest that the species from the springbuck is identical with the species recovered from from sheep and goats.

It was the recovery of oesophagostomes belonging to this subgenus from the caecum and colon of a Kudu and a Red Duiker that induced me to examine collections of nodular worms from cattle in the tropics and subtropics. Hitherto I have failed to recover oesophagostomes of this group from cattle or sheep in N. Rhodesia. The failure must be attributed partly to the fact that the material examined was from animals in localities where antelopes are no longer plentiful.

The specimens from the Kudu seem to be identical with *Oes.* (*Hudsonia*) *walkeri* recorded from the Eland. The three specimens from the Red Duiker possess a buccal capsule almost identical with that of *Oes.* (*Hudsonia*) *multifoliatum* of cattle but the most matured ova in the uteri of the two females measure only 0·07 to 0·08 mm. by 0·05 to 0·56 mm. The cephalic inflation is as recorded for the species from the Eland and the Springbuck.

The species from the Red Duiker has a buccal capsule of much less depth (14 to 17μ) than those of the specimens from the Kudu (19 to 24μ), the Eland (26μ) and Thomson's gazelle (20–23μ). The name *Oesophagostomum* (*Hudsonia*) *roscoei* is proposed, in honour of Mr. C. P. Roscoe who had the Kudu and the Duiker shot for me on his farm, for the species from the Red Duiker.

The various measurements of the specimens referred to above are recorded in table I.

The type specimens of *Oes.* (*Hud.*) *roscoei* have been deposited with the British Museum (Natural History), London.

Hudsonia. N. Subgenus.

The members of this subgenus are characterised by the possession of a cephalic inflation which is best developed ventrally and least developed dorsally. Additional characters are :—Circular oral aperture. Relatively deep circular buccal capsule with walls of medium thickness. Internal and external leaf-crown present with an equal number of elements in each crown. Circumoral papillae project hardly beyond surface of mouth

TABLE I.

The Measurements (in micro-millimetres except where otherwise stated) in members of the subgenus *Hudsonia* from domesticated stock and antelopes.

Species	*O. multifoliatum*	*O. walkeri*	*O. africana*	*O. multifoliatum*	*O. walkeri* (?)	*O.* (*Hudsonia*) *roscoei*	*O. walkeri* (?)
Authors	Daubney & Hudson	Mönnig	Mönnig	Leroux	Leroux	Leroux	Leroux
Hosts	Sheep & goats	Eland	Springbuck	Cattle	Kudu	Duiker	Thomson's gazelle
Localities	Kenya Colony	Kenya Colony	Orange Free State	Tanganyika S. Rhodesia	N. Rhodesia	N. Rhodesia	East Africa
Length of body ♂	12–14 mm.	12–35 mm.	12·7–13·72mm.	11–12 mm.	13·8 mm.	12·5 mm.	—
,, ,, ,, ♀	14–17 mm.	18·3–20·3	13·07–19mm.	14–16 mm.	16·3 mm.	16 & 18·5	—
Max. width of body ♂	400	256	410	300	288	376	—
,, ,, ,, ♀	480	420	420–590	300–350	352	495 & 450	515
Length of oesophagus ♂ ♀	900–1000	820	800–820	855	800	915	—
Max. width of oesp. anteriorly ♂ ♀	—	910–970	880–920	825–885	912	1020 & 917	915
,, ,, ,, ,, posteriorly ♂ ♀	—	—	—	87	88	100	—
Min. width of oesp. anteriorly ♂ ♀	—	—	—	84–90	100	117–120	124
,, ,, ,, ,, ,, ♂ ♀	—	—	—	80	66	87	—
Anterior end to excretory pore ♂ ♀	—	—	—	75–79	74	93–112	180
,, ,, ,, ,, ♂ ♀	—	—	—	165	136	120	—
,, ,, ,, cervical papillae ♂ ♀	200–280	200		150–180	153	183–165	225
,, ,, ,, ,, ,, ♂ ♀	370–400	260–270	170–200	270	208	210	—
,, ,, ,, nerve ring ♂	—	244		280–285	240	210–240	—
,, ,, ,, ,, ♀	—	310–320	217–244	328	288	247	—
Head : width ♂	—	300–340	260–280	300–315	295	300–368	345
,, ,, ♀	—	142		270	320	225	—
Head height ♂	—	169	100–124	280–285	368	300	—
,, ,, ♀	—	—	—	120	144	147	—
Buccal capsule. Height dorsally ♂	—	—	—	105–130	168	160–166	165
,, ,, ,, ♀	—		—	50	52	46	—
,, ,, ventrally ♂	—		—	60–66	64	50–66	52
,, ,, ,, ♀	—		—	16–17	19	14	—
,, ,, Inner width anteriorly ♂	—	26	—	16–17	19–20	14 & 15	20
,, ,, ,, ♀	—		—	18–20	23	16	—
,, ,, ,, posteriorly ♂	—		—	18–20	23–24	17 & 17	23
,, ,, ,, ♀	—	—	—	50	60	73	—
Length of spicules	750–850	787	750–840	56	63	77	74
Vulva to anus	550–600	? 627–720	—	50	60	73	—
Anus to tip of tail	260–300	270–310	290–348	56	63	77	76
Ova in uterus : length	150–165	100	150	600–630	768	790	—
breadth	68–80	56	75	525–585	560	630 & 600	570
				—	288	360 & 370	300
				120–135	83–92	70–80	84–88
				70–75	43–54	58–60	—

collar. Oesophageal funnel moderately developed and without oesophageal teeth with its cuticular lining well developed and constricted slightly from the well developed cuticular lining of the rest of oesophagus. Mouth collar well developed and marked off from rest of body by a deep cephalic groove. Cervical groove best developed ventrally and extending to lateral aspects. Prominent cervical cuticular flap forming posterior limit of cephalic inflation. Prominent lateral alae, pierced on a level with the nerve ring by the cervical papillae, extend posteriorly for almost the complete length of the body and increasing again in height on level with genital opening. Tail of female long and tapering to a fine point with caudal papillae present. Copulatory bursa with the rays relatively more slender than in the type species of the genus. Gubernaculum and genital cone of the general type. Parasites of the caecum and first portion of the colon of ruminants.

Type species :—

Oesophagostomum (*Hudsonia*) *multifoliatum* (Daubney and Hudson, 1932) parasitizing sheep, goats and cattle.

Other species :—

Oesphagostomum (*Hudsonia*) *walkeri* (Mönnig 1932) parasitizing the Eland, Kudu and Thomson's gazelle.

Oesophagostomum (*Hudsonia*) *roscoei* sp. n.

Pukuia. N. Subgenus.

Head truncated cone-shaped. Mouth collar well developed with its posterior limit overhanging a well defined cephalic groove which demarcates the head from the rest of the body. Oral aperture circular in outline. Circumoral papillae project hardly beyond the cuticle and

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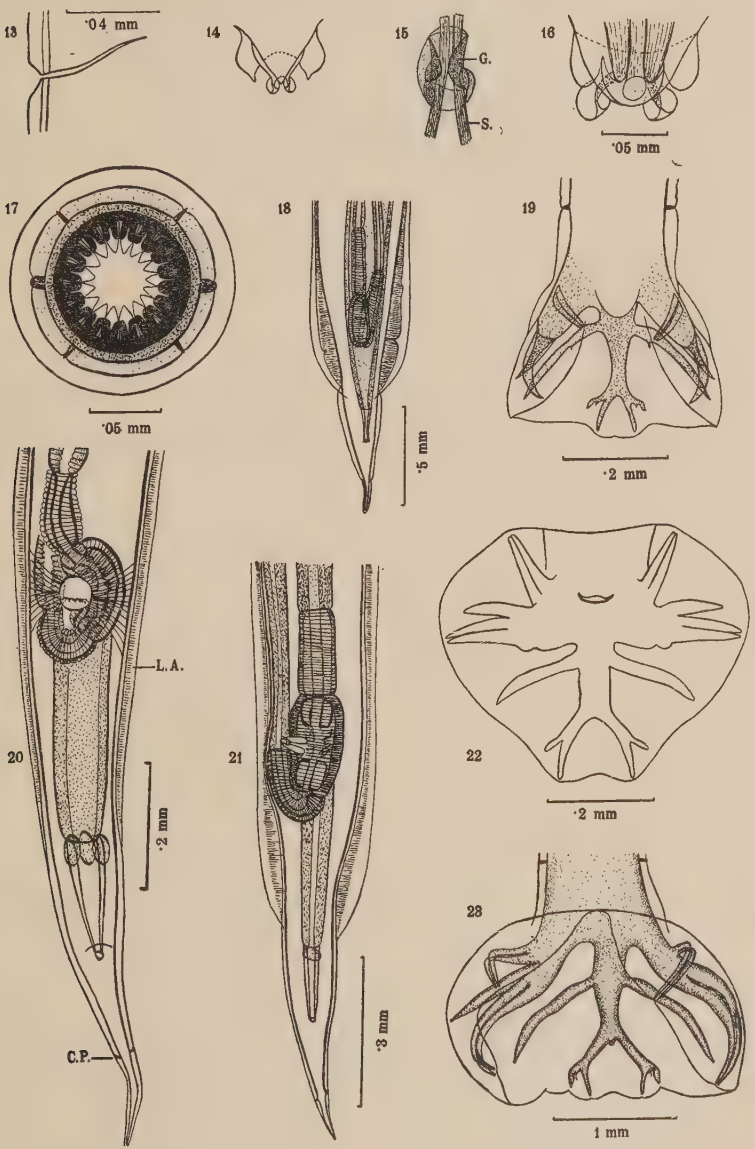
Fig. 13.—Cervical papilla : *Daubneyia oldi*.
Fig. 14.—Ventral view of genital cone : *Oes.* (*Hud.*) *roscoei*.
Fig. 15.—Ventral view of gubernaculum : *Oes.* (*Puk.*) *lechwei*.
Fig. 16.—Ventral view of genital cone : *Oes.* (*Puk.*) *lechwei*.
Fig. 17.—Anterior view of oral aperture : *Oes.* (*Puk.*) *lechwei*.
Fig. 18.—Ventral view of caudal end : *Oes.* (*Hud.*) *roscoei*.
Fig. 19.—Copulatory bursa : *Oes.* (*Puk.*) *lechwei*.
Fig. 20.—Caudal extremity : *Oes.* (*Puk.*) *lechwei*.
Fig. 21.—Caudal extremity : *Oes.* (*Hud.*) *multifoliatum*.
Fig. 22.—Copulatory bursa : *Daubneyia* type.
Fig. 23.—Copulatory bursa : *Oes.* (*Hud.*) *roscoei*.

located on lateral aspects of the head. Cephalic inflation slightly developed. Cervical groove well marked ventrally and extending halfway



on to lateral surfaces. Excretory pore situated in cervical groove which

is overlapped by a cuticular flap. Well developed lateral alae, originating from the termination of the cervical cuticular fold at mid lateral lines, extend posteriorly for almost the whole length of the body. Prominent cervical papillae pierce lateral alae on a level just posterior to the cervical groove. Buccal capsule circular, of medium height and with thick wall. Elements of external leaf-crown relatively long and broad. Elements of internal leaf crown long, leaf-crowns prominent and with an equal number of elements in each. Elements of external leaf-crown relatively long and broad. Internal leaf-crown elements very long, broad anteriorly thickened latero-medially directed anteriorly and medially, inner surface concave in both directions causing element to appear grooved medially in an *enface* view. In a latero-medial view there appear two highly refractive rod-like structures at the base of the external element. Vagina short. Caudal extremity of female terminating in long attenuated tail. Copulatory bursa, genital cone, spicules and gubernaculum of general *Oesophagostomum* type. Parasite inhibiting the caecum and first few feet of colon of ruminants.

Type species :—

Oesophagostomum (*Pukuia*) *lechwei* sp. n.

Type host :—

Puku (*Adenota vardoni* syn. *Cobus vardoni*).

The type and some cotype specimens have been deposited with the British Museum (Natural History) South Kensington, London.

Cotypes have also been deposited with the Helminthology Department, London School of Hygiene and Tropical Medicine.

Morphology of *O.* (*Pukuia*) *lechwei*.

The general characters of this species have been stated in the diagnosis of the subgenus. The chief measurements of the males and females measured are tabulated. (Table II.)

The freshly collected specimens were of a rather dark dirty grey colour and this rendered their detection amongst the faecal matter of the large intestines rather difficult. Specimens washed in normal saline and preserved by dropping them into a boiling mixture of ten per cent. glycerine in 70 per cent. alcohol, retained their dark colour which was due to the heavy pigmentation of the intestinal cells.

The anterior extremity of the worm, in both unpreserved and preserved specimens, was bent dorsally and not ventrally as recorded for oesophagostomes furnished with well developed lateral alae.

The external leaf-crown is composed of seventeen to twenty relatively long and stout elements. The internal leaf-crown is composed of a similar number of elements as found in the internal leaf-crown. In a latero-medial view the number of elements appeared to be double the number of elements in the external crown and there was a wide space

TABLE II.

The various measurements (in micro-millimetres except where otherwise stated) in the species *Oesophagostomum* (*Pukuia*) *lechwei* were :—

	Males.	Females.
Length of body	7 mm. to 8·5 mm.	9·5 mm. to 11·5 mm.
Maximum width of body	223–255	295–320
Length of oesophagus	675–720	825–945
Max. width of oesophagus anteriorly ...	105–120	120–135
Max. width of oesophagus posteriorly	126–148	135–158
Min. width of oesophagus	75–87	87–99
Anterior end to excretory pore ...	220–247	240–265
,, ,, ,, cervical papillae ...	240–285	255–285
,, ,, ,, cervical groove ...	220–247	240–265
,, ,, ,, nerve ring	300–310	280–320
Head : height	70–83	70–85
width	130–158	160–167
Buccal capsule : Height dorsally ...	16–18	16–19
ventrally ...	20–22	20–23
Inner diameter	66–70	73–76
Length of spicules	645–720	—
Vulva to anus	—	525–690
Anus to tip of tail	—	285–360
Length of ovejectors : Pars ejectrix ...	—	160–170
Pars haustrix ...	—	150–167
Ova in uterus : Length	—	58–66
Breadth	—	36–40

between pairs of internal elements. When an end-on examination of the head was made it was found that the space between pairs of teeth mentioned above was in reality due to the shape of the elements as already described. In lateral view the anterior third of the element appeared thickened latero-medially. As these characters of the internal leaf elements were rather different from those mentioned in descriptions of other species it was decided to postpone the description until more specimens could be collected and all the descriptions of the various species by various authors consulted. In the case of the species described here from antelopes the leaf-crown constitutes an important part of the buccal

armature. The elements arise from about the middle of the medial surface of the wall of the buccal capsule proper. The length of the internal leaf elements is 0·025 mm. dorsally and 0·0234 ventrally. The corresponding measurements of the wall of the buccal capsule are 0·015 and 0·02 mm. The thickness of the wall of the buccal capsule is approximately 0·012 to 0·014 mm.

The rest of the characters of this species are illustrated by the accompanying figures.

This species is probably more closely related to *Oesophagostomum* (*Proteracrum*) *columbianum* than to any other known species. The build of the buccal armature is such that it could not possibly be grouped with the common nodular worm parasitizing sheep in Southern Africa.

The two new subgenera can be briefly differentiated from the other subgenera by the following key :—

1. Cuticular cervical inflation well developed.....2
   Cuticular cervical inflation less well developed.....3

2. Two leaf-crowns present.....4
   One leaf-crown present.....*Bosicola*

4. External leaf-crown elements very short and relatively numerous .....*Ihleia*
   External leaf-crown elements long and relatively few in number.....5

5. Cervical papillae close to or posterior to caudal end of oesophagus ..... *Hysteracrum*
   Cervical papillae not close to caudal end of oesophagus.....6

6. Oesophageal teeth absent.....*Oesophagostomum*
   Oesophageal teeth present.....*Conoweberia*

3. Cuticular cervical inflation much better developed ventrally and laterally than dorsally.....*Hudsonia*
   Cuticular cervical inflation poorly developed 7

7. Internal leaf-crown elements very long and stout and worms relatively small.....*Pukuia*
   Internal leaf-crown elements short and worms relatively large ..... *Proteracrum*

The species which could with certainty be assigned to the various subgenera appear to be as follows :—

I. Subgenus *Oesophagostomum* Railliet and Henry, 1913

Type-species :—*Oesophagostomum* (*Oesophagostomum*) *dentatum* (Rudolphi, 1803)

Other species :—*Oes.* (*Oes.*) *quadrispiniculatum.* (Marcone, 1901) Alicata, 1935

Oes. (*Oes.*) *brevicaudatum* (Schwartz and Alicata, 1930)

Oes. (*Oes.*) *georgianum* (Schwartz and Alicata, 1930)

Oes. (*Oes.*) *maplestonei* (Schwartz, 1931)

II. Subgenus *Proteracrum* Railliet and Henry, 1913

Type-species :—*Oesophagostomum* (*Proteracrum*) *columbianum* (Curtice, 1890) R. & H., 1913

III. Subgenus *Hysteracrum* Railliet and Henry, 1913

Type-species :—*Oesophagostomum* (*Hysteracrum*) *venulosum* (Rudolphi, 1809) Railliet and Henry, 1913

Other species :—*Oes.* (*Hyst.*) *asperum* Railliet and Henry, 1913

Oes. (*Hyst.*) *indicum* (Maplestone, 1931) Travassos and Vogelsang, 1932

Oes. (*Hyst.*) *vigintimembrum* (Canavan, 1931) Travassos and Vogelsang, 1932

(?) *Oes.* (*Hyst.*) *okapi* (Leiper, 1935)

IV. Subgenus *Conoweberia* Ihle, 1922

Type-species :—*Oesophagostomum* (*Conoweberia*) *bifurcum* (Creplin, 1849) Travassos & Volgelsang, 1932. = *Oesophagostomum* (*Conoweberia*) *apiostomum* (Willach, 1891) Ihle, 1922.

Other species :—*Oes.* (*Conow.*) *pachycephalum* (Molin, 1891) Travassos and Vogelsang, 1932

Oes. (*Conow.*) *aculeatum* (v. Linstow, 1879) Travassos and Vogelsang, 1932

Oes. (*Conow.*) *ovatum* (v. Linstow, 1906) Travassos & Vogelsang, 1932.

Oes. (*Conow.*) *blanchardi* (Railliet and Henry, 1912) Travassos and Vogelsang, 1932

Oes. (*Conow.*) *xeri* (Ortlepp, 1922) Travassos and Vogelsang, 1932

Oes. (*Conow.*) *susannae* (Leroux, 1929) Travassos and Vogelsang, 1932

V. Subgenus *Ihleia* Travassos and Vogelsang, 1932
Type-species :—*Oesophagostomum* (*Ihleia*) *stephanostomum* (Stossich, 1904) Travassos and Vogelsang, 1932
Other species :—*Oes.* (*Ihleia*) *ventri* (Thornton, 1924)

VI. Subgenus *Hudsonia* N. Subg.
Type-species :—*Oesophagostomum* (*Hudsonia*) *multifoliatum* (Daubney and Hudson, 1932)
Other species :—*Oes.* (*Hudsonia*) *walkeri* (Mönnig, 1933)
Oes. (*Hudsonia*) *roscoei* sp. n.

VII. Subgenus *Pukuia* n. subg.
Type-species :—*Oesophagostomum* (*Pukuia*) *lechwei*. sp. n.

VIII. Subgenus *Bosicola*
Type-species :—*Oesophagostomum* (*Bosicola*) *radiatum* (Rud., 1803) Travassos and Vogelsang, 1932
Other species :—*Oes.* (*Bosicola*) *curvatum* (Maplestone, 1931) Travassos and Vogelsang, 1932
Oes. (*Bosicola*) *traguli* (Chandler, 1931)

The species *Oesophagostomoides traguli* Maplestone, 1932 had been transferred to the genus *Oesophagostomum* by Baylis (1936). Maplestone's description of this species does not allow of it being grouped in any of the above named genera. The length of the specimens shows them to be rather minute helminths. A more detailed description of this species is desirable.

The species *Oesophagostomum tridentatum* Maplestone, 1932 from the stomach of the Dusky Langur (*Semnopithecus obscurus*) has a rather deep and atypical buccal capsule. The build of the buccal capsule suggests affinities with *Ternidens deminutus*. It may be an oesophagostome but shows no real relationship to any of the groups.

Oesophagostomum (*Conoweberia*) *zukowskyi* Travassos and Vogelsang, 1931 from *Papio* (*Maimon*) *maimon* is another species with a rather deep and atypical buccal capsule and seems related to *O. tridentatum*.

Travassos and Vogelsang's illustrations of the species *Oes.* (*Ihleia*) *stephanostomum* from *Gorilla gorilla* and *Anthropopithecus troglodytes* suggest that the helminths from these two hosts may belong to different species. The oesophagus in the case of Figs. 182 and 183 is only about half the length of the oesophagus in Figs. 189 and 190.

The Oesophagostomes of the Wart-Hog.

The oesophagostomes recorded from the Wart-hog (*Phacochoerus aethiopicus*) are described as having a well developed external leaf-crown but no internal crown. I had the opportunity of examining some of the species during my stay in London and have to conclude that they are not true oesophagostomes and should not be retained in the genus *Oesophagostomum* Molin, 1861. The new genus *Daubneyia* is here proposed for their reception. The specimens examined by me were from the collection originally obtained from the Wart-hog on the Gold Coast. This material was by no means well preserved but a few reasonably good specimens were available. Amongst the best preserved were some specimens of the species *mwanzae, oldi* and *yorkei* without a ventral groove, while the poorer specimens showed a groove as well as one or more smaller and more anteriorly placed grooves. The condition of the specimens suggests that they were kept in water before being preserved in hot alcohol because some of the specimens of *D. yorkei* (Thornton, 1924) were ruptured, as happens when the smaller species of *Trichonema* are kept in ordinary water. Some of the worms had a small oxyurid embedded in the mucus and faecal matter adhering to them. This oxyurid seems morphologically identical with *Probstmayria vivipara* (Probstmayr, 1865) Ransom, 1907. This species was collected in 1929 by the writer from a Wart-hog in Zululand, Natal.

The absence of a definite ventral groove and lateral alae and the presence of rather long slender cervical papillae which may often be broken off suggest that the oesophagostomes of the Wart-hog belong to a genus or genera which should be included in the subfamily Trichoneminae Railliet, 1916. Microscopically these worms resemble those of the genera *Trichonema* Cobbold, 1874 and *Murshidia* Lane, 1914. As in the last mentioned genus the mouth collar is lower dorsally and ventrally than laterally. The sub-dorsal and sub-ventral cephalic papillae are long, the cervical papillae very long and slender and the wall of the buccal capsule is thick posteriorly and bluntly pointed anteriorly. The genus *Bourgelatia* appears according to Maplestone's (1930) description of *B. diducta* to be more closely related to the subfamily Oesophagostominae Railliet, 1915 than to the subfamily, where it has been grouped.

The chief characters of the genus *Daubneyia* may be briefly defined as follows :—

Anterior end with or without cuticular inflation. External leaf-crown, composed of six to eight elements, present. Mouth collar markedly depressed dorsally and ventrally causing the formation of two lateral "lips." Subdorsal and subventral cephalic papillae rather long. Cervical papillae long and slender. Prebursal papillae short and not readily detected. Buccal capsule thick-walled and not wholly situated in mouth collar. Posterior extremity of female with relatively short, bluntly pointed tail which may or may not be tilted dorsally. Copulatory bursa with lobes distinct and rays rather stout.

As type of the genus is selected *Daubneyia mwanzae* (Daubney, 1924) which would appear to be the most common species.

Other species are :—*Daubneyia oldi* (Goodey, 1924)
Daubneyia yorkei (Thornton, 1924)
Daubneyia goodeyi (Daubney, 1926)
Daubneyia roubaudi (Daubney, 1926)
Daubneyia simpsoni (Goodey, 1924)
Daubneyia eurycephalum (Goodey, 1924)

The species *Daubneyia mwanzae*, *D. simpsoni*, *D. oldi* and *D. eurycephalum* are recorded by Goodey from the Roan Antelope in Nyasaland. I doubt whether the worms were really collected from that host. All the species mentioned above have been recorded from the Wart-hog. The species *D. goodeyi* and *D. roubaudi* have been rather briefly described. A re-examination of the type and co-types and of the collections examined by Daubney, Goodey, and Thornton would seem to be indicated because the various measurements of *D. mwanzae* given by Daubney (1924), Goodey (1924) and Daubney (1926) suggest that more than one species may have been included.

Finally it may be noted that Travassos and Vogelsang (1932, p. 304) list *Oes.* (*Prot.*) *columbianum* as a parasite of the Wart-hog. This is surely not correct. In the wild state the Wart-hog does not seem to eat dead meat while the bush pig does. The inclusion of the Wart-hog in the family Suidae does not seem quite correct. I have been assured that the Wart-hog is immune to East African Swine Fever, while the bush pigs are very susceptible. Some natives consider the Wart-hog a "small brother" of the rhinoceros. They consider him akin to the rhinoceros in some of his habits.

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On *Anguillulina multicincta* (Cobb) and Other Species of *Anguillulina* associated with the Roots of Plants.

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Since the publication of the writer's (1932) paper on the nematode genus *Anguillulina*, opportunities have occurred from time to time for making further observations on the morphology and anatomy of some of the species of the genus found associated with the roots of plants. Certain of them are parasitic in roots, at least during part of their life, at other times occurring free in the soil. Others have not been found within roots but have been obtained only in water extracts of turf and their exact relation to the roots of plants is at present undetermined.

Some nematodes obtained from banana roots have been identified with *Anguillulina multicincta* (Cobb) which is herein re-described and figured. As a consequence of this it has become necessary to differentiate, under the name of *Anguillulina erythrinae* (Zimmermann), a widely distributed species which in the 1932 paper was considered to be synonymous with *A. multicincta*. The species is re-described and figured. Further observations are presented on *Anguillulina robusta*, including an account of the male, and *A. obtusa*, the male of which is adequately described and figured for the first time.

Anguillulina multicincta (Cobb, 1893).
syn. *Tylenchus multicinctus* Cobb, 1893.
nec *Anguillulina multicincta* of Goodey, 1932.

Cobb based his description and figures of this species on adult specimens obtained from about the roots of banana plants growing in Fiji. The worms studied by the writer were obtained from lesions in banana rootlets originating from the vicinity of Apia, Samoa. The material, preserved in formalin, was sent to the writer by the Imperial Mycological Institute for a diagnosis of the nematodes present. The lesions in which the nematodes were found were very small reddish brown areas lying

immediately below the surface of some of the rootlets and in these occurred adult males and females and larvae of the worms. A single female of this species was also found in diseased banana root material (*Musa Cavandishii*) from Guadeloupe, French West Indies.

Adults of this species show so close a resemblance in size, shape and anatomical detail to the worms described by Cobb that the writer has not hesitated to identify them with Cobb's species.

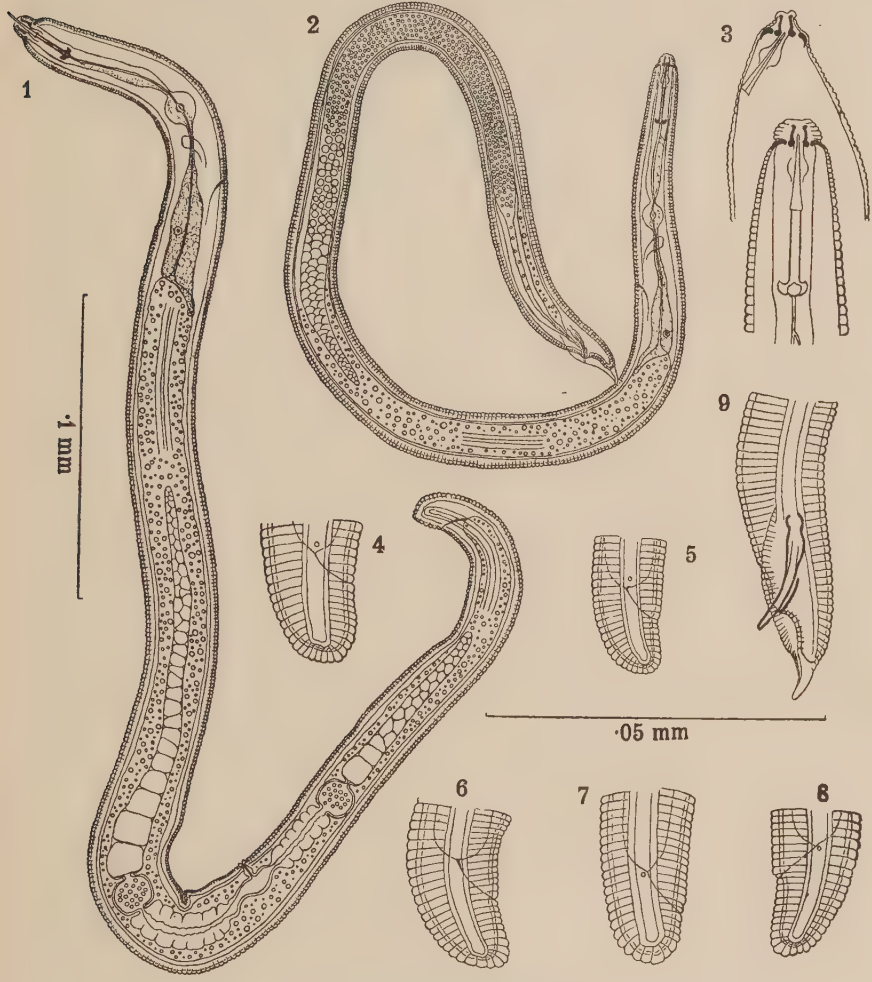
Morphology.

Dimensions: *Female*, length, 0·463 mm. to 0·68 mm., α=23·8–28·5, β=4·5–6, γ=48·1–63, V=64·6%–71·8%. Average values based on 10 specimens, length, 0·546 mm., α=24·47, β=4·81, γ=53·8, V=69·13%, buccal spear, 20μ–24μ. *Male*, length, 0·435 mm. to 0·556 mm., α=27·2–32·7, β=3·76–4·8, γ=28·35·6. Average values based on 8 specimens, length, 0·486 mm., α=28·8, β=4·3, γ=31·7, spicules, 20μ–22μ, gubernaculum, 6μ–7μ.

Cobb gave 0·5 mm. as the length of both male and female. Body tapering but little anteriorly and posteriorly. Cuticle rather thick and showing well defined transverse striae. Lateral fields about one third width of body. Head roundly conical; offset by narrow constriction and carrying transverse striae. Cuticular framework of head consisting of central tubular vestibule, the ends of the tube being rather knobbed. Lower ends connected on either side to transverse crescentic elements at level of head constriction and continued centrally into a lyre-shaped spear guide. Buccal spear 20μ to 24μ long, the three basal swellings well developed and concave on their anterior faces. During ecdysis the anterior conical half of the spear is shed along with the cuticular head framework and the lyre-shaped spear guide (fig. 3). Oesophagus typical; the muscular bulb generally situated about halfway down but sometimes a little more posteriorly placed. Isthmus narrow and then expending into the rather elongate and irregularly shaped posterior swelling containing the three oseophageal gland cells. Nucleus of dorsal gland cell generally easy to locate but nuclei of two sub-ventral gland cells not clearly distinguishable. Intestine well stocked with fatty food globules, connected with anus by distinct rectum.

Female.—Tail tapering very slightly; in some examples scarcely at all, rather short, consisting of from 8 to 11 transverse striae. Ventral side more or less a continuation of straight line of body; curvature of tail

coming from dorsal side (figs. 4–8). Tip of tail rather broad and bluntly rounded. The final striae on ventral side not especially prominent and



Anguillulina multicincta (Cobb).

Figs. 1 & 2.—Adult female and male, showing general shape and structure. Scale on left applies to both.

Fig. 3.—Head end highly magnified to show shape of head framework and spear and structures shed during ecdysis.

Figs. 4–8.—Female tails, highly magnified, in lateral aspect, to show shape of tip of tail.

Fig. 9.—Male tail, highly magnified, in lateral aspect, to show bursa and shape of spicules and gubernaculum. Horizontal scale applies to figs. 3–9.

exhibiting but little tendency to form a pointed process. A small papilla present on either side of body in middle of lateral field and situated pre-anally ; occasionally absent from one side of body as in fig. 6. Vulva roughly at two-thirds body length from anterior end. In 10 specimens in which the percentage distance of the vulva from the anterior end was accurately determined it varied from 64·6% to 71·8%, the average value being 69·13%. Cobb gave V=66% ; the writer's determinations therefore agree fairly well with Cobb's value. Gonads paired, opposed and outstretched ; a spherical receptaculum seminis lying between each uterus and ovary. One egg at a time in each ovary.

Male.—Body tapering slightly from a short distance in front of the cloacal aperture to tip of tail. Latter completely enclosed by bursa, free edge of which is crenate corresponding to fine striations of bursal wings. Lateral caudal papillae absent. Spicules paired, arcuate and shaped as in fig. 9. Anterior end rounded and cephalated by constriction from shaft which gradually tapers to moderately sharp but rounded points. Gubernaculum simple. Gonad single, outstretched and reaching a little more than halfway to end of oesophagus ; posterior quarter with stouter walls and forming vas deferens. Small round spermatozoa very numerous in middle region in some specimens.

Occurrence.

Parasitic in rootlets of banana (species uncertain) from Samoa and *Musa Cavendishii* Lambert (dwarf banana) from Guadeloupe, French West Indies.

Discussion.

The identity of the nematodes with those described by Cobb is supported by the fact that they were found associated with the same host plant and from the same quarter of the globe. Another fact worthy of note is that adult males were practically as abundant as females in the lesions. With regard to the shape of the spicules, Cobb said that the proximal ends were not cephalated whereas the writer has found them to be knobbed. Cobb's drawings of the male tail, and of the spicules, however, agree well with those of the writer and one cannot but assume that in interpreting the shape of the spicules Cobb was in error. The differentiation of this species from *A. erythrinae* is dealt with further in the discussion on that species.

Anguillulina erythrinae (Zimmermann, (1904).

syn. *Tylenchus erythrinae* Zimmermann, 1904.
Tylenchus olaae Cobb, 1906.
Tylenchus pseudorobustus Steiner, 1914.
Aphelenchus dubius var. *peruensis* Steiner, 1920.
Tylenchus spiralis Cassidy, 1930.
Tylenchorhynchus robustus var. *erythrinae* (Zimmermann, 1904). Bally & Reydon, 1931.
Anguillulina multicincta Goodey, 1932.
Tylenchorhynchus multicinctus (Cobb) Schuurmans Stekhoven & Teunissen, 1938.

Considerable numbers of adult females of this species have been obtained in water extractions, by the Baermann funnel technique, from turf samples taken from pastures at this Institute, from lawns and pastures at Beckenham in Kent and from a piece of turf from a bowling green at Bath. Adult females from a coffee nursery soil in south India have also been examined and found to be identical with specimens of English origin. Adult males along with egg-bearing females have also been obtained in good numbers from the roots of the common grass, *Agrostis stolonifera*, L., after staining in acid fuchsin-lactophenol ; the worms and eggs laid by the females being fairly abundant in the cortex of the roots in November and December. Males of this species occur much less frequently than females in water extracts of turf. At the time the 1932 paper was published the writer had not found a single male specimen. Between that time and the autumn of 1939 three examples only had been found whereas good numbers of females had been obtained from turf at various times of the year. In October, 1939, however, in an extract from a piece of turf mainly consisting of *Agrostis stolonifera* taken from a rough pasture at this Institute 22 males were obtained.

Morphology.

Dimensions : *Female*, length, 0·61 mm. to 0·92 mm., α=24–32, β=4·6–6·5, γ=41–64, V=59%,–65%, buccal spear, 24μ–25μ. Average values based on 55 specimens, length, 0·75 mm., α=29·5, β=5·4, γ=49·6, V=63%. *Male*, length, 0·565 mm. to 0·8 mm., α–25–36·8, β=3·76–4·68, γ=28·2–36, spicules, 25μ–27μ, gubernaculum, 6μ–7μ. Average values based on 25 specimens, length, 0·654 mm., α=31·1, β=4·37, γ=29·6.

As can be seen by comparing the drawings of this species with those of *A. multicincta*, the adults resemble each other fairly closely in appearance and structure and it is therefore necessary only to indicate the chief differences between them.

1. *Size.*—Adults of both sexes are on the whole larger than those of *A. multicincta*; the smallest males and females of *A. erythrinae* being about as long as the longest males and females, respectively, of *A. multicincta*.

2. *Female Tail.*—The shape of the female tail in *A. erythrinae* is different from that of *A. multicincta* as can be seen from a comparison of figs. 4–8 with figs. 13–20, which are all drawn to the same scale. Figs. 13–20 show that the tail of *A. erythrinae* exhibits a considerable range in the extent to which it forms a ventral terminal process. It is also apparent from these drawings that the presence of a distinct process depends on the extent to which the last few transverse striations of the body are elongated posteriorly. The drawings also show that the lateral caudal papillae are somewhat variable in position. Most commonly they are pre-anally situated but occasionally, as in figs. 19 and 20, they are practically ad-anal.

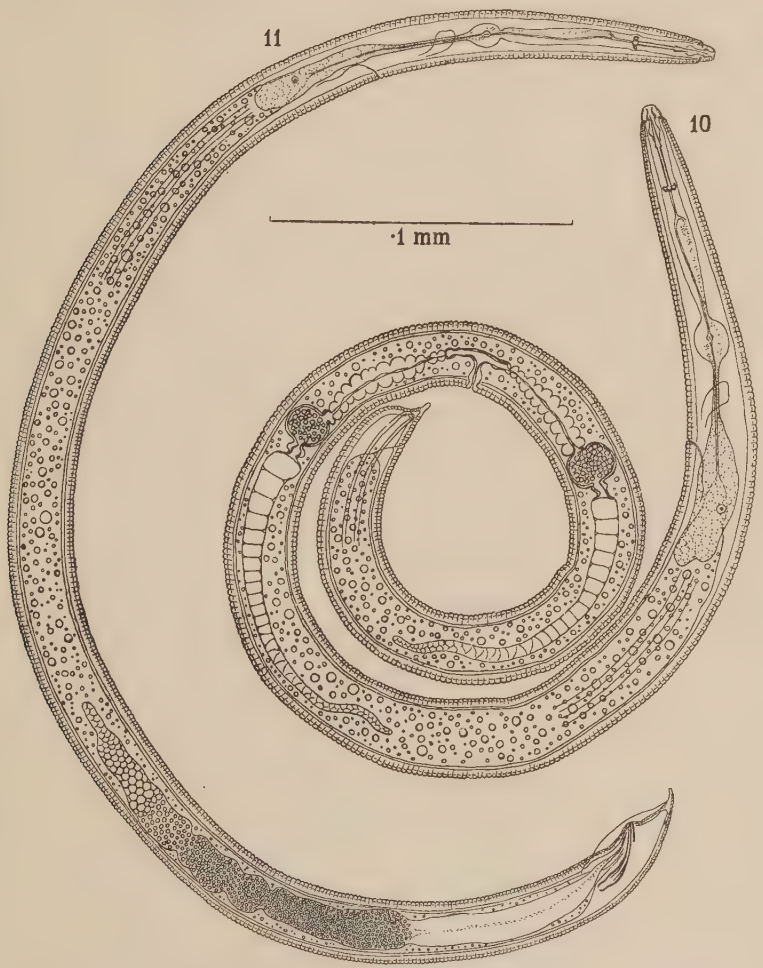
3. *Position of Vulva.*—In *A. multicincta* the vulva is situated relatively further back than in *A. erythrinae.* In 10 specimens of the former the value of V varied from 64·6% to 71·8%, the average being 69·13%. In 23 specimens of *A. erythrinae* from Winches Farm turf, varying in length from 0·633 mm. to 0·775 mm. the value of V varied from 58·3% to 63·5% the average value being 61·2%. In the case of 55 females from Beckenham turf ranging in length from 0·61 mm. to 0·92 mm., V varied from 60% to 65%, the average value being 63%.

In these three features, i.e., greater length, different shape of female tail and relative position of the vulva, the writer considers that *A. erythrinae* can be specifically differentiated from *A. multicincta.* To these points may also be added the larger size of the male with correspondingly larger and more heavily built spicules and gubernaculum. The male of *A. erythrinae* like that of *A. multicincta* has no lateral caudal papillae within the bursa.

Occurrence.

The species is parasitic in habit. It was reported by Zimmermann from the roots of dadap, *Erythrina lithosperma* Blume, and the writer has found adult males and female eggs and larvae within the cortex

of the roots of the common grass, *Agrostis stolonifera* L., and larval females in the same situation in the roots of oat seedlings, *Avena sativa* L.



Anguillulina erythrinae (Zimmermann).

Figs. 10 & 11.—Adult female and male showing general shape and structure.

The worms do not lie parallel to the root axis as do the adults of *A. pratensis* and *A. obtusa* for the most part, but seem to produce cavities in the cortex within which they lie more or less freely coiled and from which

it is possible to dislodge them comparatively easily by means of fine needles. Whether they have a harmful effect on these hosts cannot be stated with certainty as the grasses and oats examined showed no evident symptoms of disease. Linford (1939) states that he has found this species to feed freely in the roots of various plants which, however, he does not name.

Systematics.

The separation of the species *A. multicincta* occurring in banana roots from the more widespread and larger species, now named *A. erythrinae*, necessitates a brief discussion on the choice of the specific name *erythrinae* and on the question of synonymy. The worms described by Zimmermann under the name *Tylenchus erythrinae* appear to be morphologically identical with those described in the present paper. The length of the body 0·56 mm. to 0·7 mm., the shape of the female tail with a terminal ventral process and the relative position of the vulva (V=60%) are features which may be specially mentioned. Since Zimmermann's is the earliest description of the species known to the writer his specific name must be retained for it.

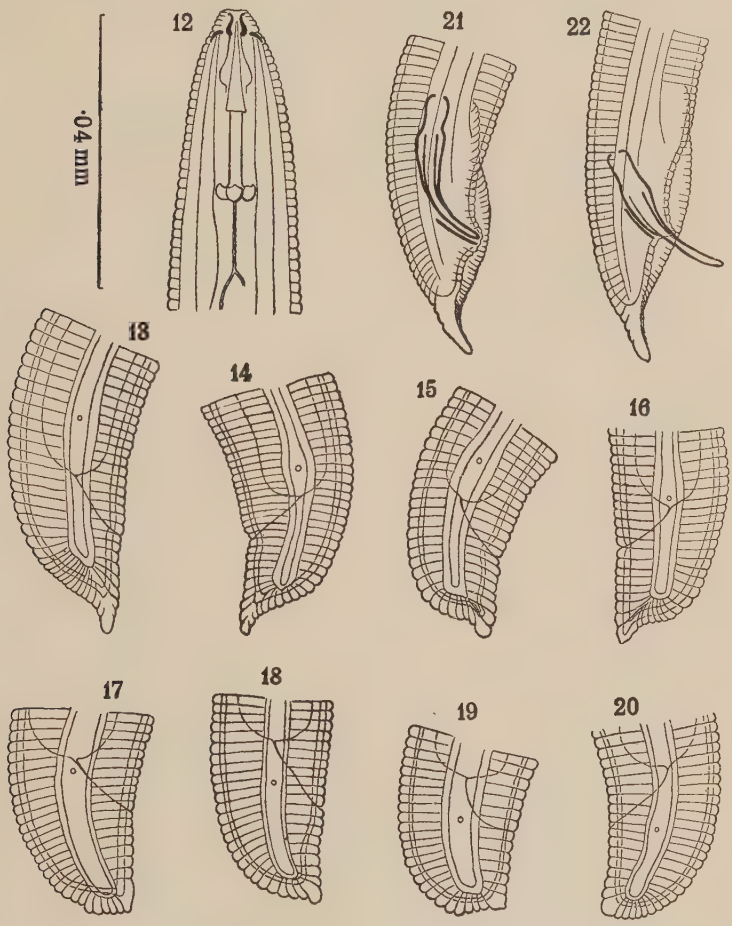
Tylenchus olaae Cobb, 1906 is tentatively included as a synonym because it has about the right length, 0·72 mm., and because the proportions α=25·6 and β=5·75 and V=63% agree pretty well with those found by the writer. The shape of the female tail is described by Cobb as " convex conoid, to the rather blunt terminus " from which one may perhaps infer that there is no terminal process. Unfortunately Cobb gave no drawings of this species which would have enabled one to settle the point.

Tylenchus pseudorobustus Steiner, 1914 appears to be valid synonym for though only an immature female was described by Steiner without dimensions his drawings of the head and tail show that the worm most probably belongs to this species.

Aphelenchus dubius var. *peruensis* Steiner, 1920 of which only a single female was found appears to be a true synonym since it agrees in size, shape of head and tail and in the position of the vulva.

Tylenchus spiralis Cassidy, 1930 and *Tylenchorhynchus robustus* var. *erythrinae* (Zimmermann, 1904) Bally & Reydon, 1931 are clearly synonyms since the published dimensions and drawings of them agree in practically all points with those of *A. erythrinae*.

Tylenchus africanus Micoletzky, 1915 though included as a synonym of this species in the writer's 1932 paper is excluded at the present time



Anguillulina erythrinae (Zimmermann).

Fig. 12.—Head end, highly magnified, in lateral aspect.
Figs. 13–20.—Female tails, highly magnified, in lateral aspect, to show variable character of terminal ventral process and relative position of lateral caudal papillae.
Figs. 21 & 22.—Male tails, highly magnified, in lateral aspect. The left upper scale applies to all figs.

since Schuurmans Stekhoven & Teunissen (1938) have recently shown that the form described by them and which they identify with Micoletzky's

T. africanus has a sharply conical tail which ends in a terminal, not ventral, process. They consider it to be distinct from the nematodes which they describe and figure under the name of *Tylenchorhynchus multicinctus* (Cobb) which, from their drawings (fig. 6) is clearly identical with *A. erythrinae* of the present paper.

ANGUILLULINA ROBUSTA (de Man, 1876) Goodey, 1932.

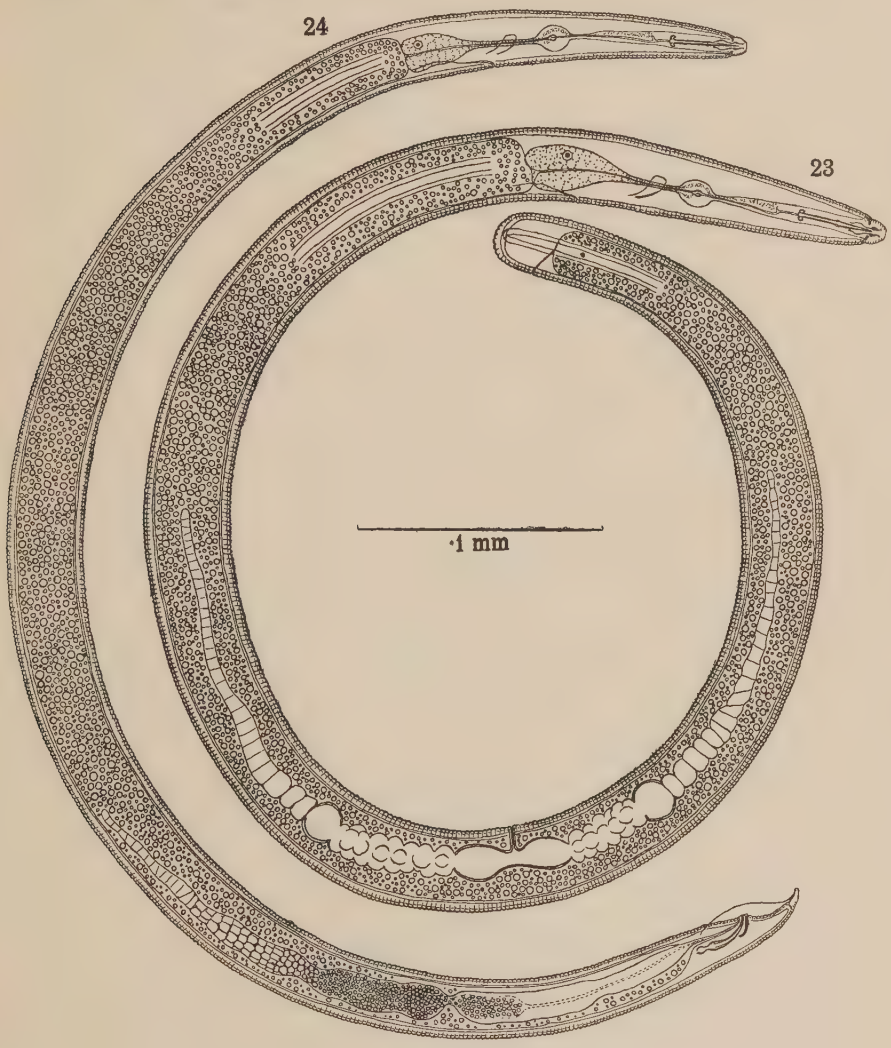
syn. *Tylenchus robustus* de Man, 1876.
Tylenchus dihystera Cobb, 1893.
Tylenchorhynchus robustus v. *brevicaudatus* Micoletzky, 1921.
Rotylenchus robustus (de Man, 1876) Filipjev, 1936.

MORPHOLOGY.

Dimensions : *Female*, length, 0·88 mm. to 1·18 mm., α=24–32·2, β=5·8–7, γ=40–65, V=52%–61·5%, buccal spear, 28μ–30μ. Average values based on 14 specimens, length, 1·02 mm., α=25·75, β=6·45, γ=53, V=55·05%. *Male*, length, 0·745 mm. and 0·834 mm., α=26·8–28·6, β=5·5–6, γ=25·7–30·9, spicules, 27μ–30μ, gubernaculum,12μ–14μ. Two examples only found.

Female.—There is little to add to the description of the adult female given in the 1932 paper. The principal anatomical features are clearly indicated in fig. 23, which shows that, morphologically, this species is essentially similar to *A. multicincta* and *A. erythrinae*. The tail is short, broad and rounded and whilst exhibiting a certain amount of variation in the shape and in the arrangement of the terminal striae (figs. 26–28) there is no tendency for the final striae on the ventral side to form a pointed process. The ventral side of the tail is continuous with the ventral line of the body ; its rounded character being from the dorsal side. The lateral caudal papillae are pre-anal in position. The structure of the head and the buccal spear is the same as in *A. multicincta* and *A. erythrinae*. The spear is of slightly greater length than in those two species and the basal knobs are concave on their anterior faces. The oesophagus is typical in structure and the posterior glandular region is rather variable in the extent to which it wraps round the narrow connection between the intestine and the median oesophageal bulb. Sometimes this region is distinctly clavate in shape as in fig. 23. with the fine lumen of the oesophagus running through it centrally and finally expanding at the junction of oesophagus and intestine which is well defined. In other specimens, as

in fig. 50 of the writer's 1932 paper, the bulk of the glandular region lies rather dorsally to the anterior narrow commencement of the intestine



Anguillulina robusta (de Man).

Figs. 23 & 24.—Adult female and male showing general shape and structure. Drawn to lower scale than figs. 1 & 2 and figs. 10 & 11.

which it partly wraps round. In both cases the oesophageal gland cells lie within this part of the oesophagus and the particular arrangement of

the posterior part of the oesophagus relative to the beginning of the intestine is a very variable one upon which one could scarcely establish a diagnostic differential character. This seems to have been done by Filipjev (1936) who, in establishing his genus *Rotylenchus*, says that the oesophagus is aphelenchoid i.e., with the oesophageal glands outside its walls. Whether in species of the genus *Anguillulina* the oesophageal gland cells can ever be considered as lying outside the confines of the oesophagus seems to the writer to be altogether doubtful but in the case of *A. robusta* they certainly do not.

The vulva is slightly post-equatorial in position ; the average value of V in 14 specimens being 55·05%, i.e., it is more anteriorly placed than in *A. erythrinae* and *A. multicincta*. As in those two species the gonads are paired, opposed and outstretched and between each uterus and its ovary there occurs a spherical receptaculum seminis. The writer has never encountered egg-bearing females.

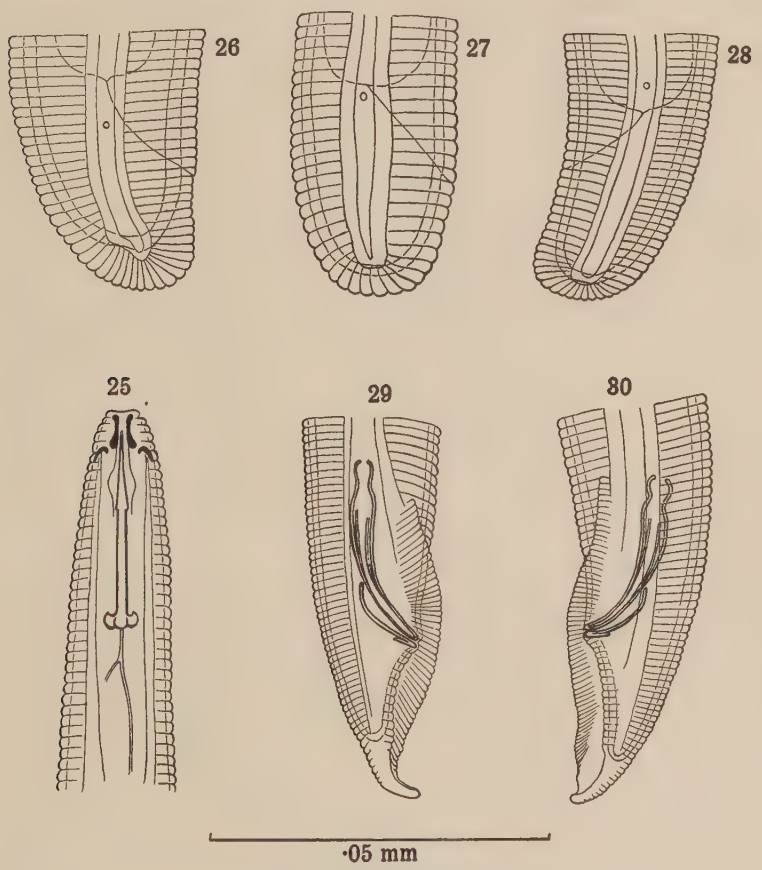
Male.—The general appearance and structure of the male is shown in fig. 24, which is a drawing of the larger of the only two specimens found by the writer. Although in both specimens the posterior region of the oesophagus was clavate in outline and did not overlap the beginning of the intestine, this anatomical feature is so variable as to be unreliable for purposes of differentiation. The main anatomical features are the same as in the male of *A. erythrinae*, i.e., gonad single and outstretched anteriorly ; bursa completely enclosing the tip of the tail and lacking any lateral caudal papillae. The spicules are rather slender and cephalated by constriction ; being a little longer than those of *A. erythrinae*. It is in the gubernaculum that the real difference between these two species is found. In *A. erythrinae* it is simple and rather short (figs. 21 & 22) whereas in *A. robusta* (figs. 29 & 30) it is longer, is turned upwards towards the spicules at its inner end whilst distally, under the points of the spicules it is thickened on the under side for a short distance with a dorsally directed point. In having no caudal papillae the males found by the writer do not agree with the male of *Tylenchus robustus* figured by de Man (1884) which is shown as having a post-anal papilla.

Occurrence.

In soil about the roots of grasses. At the present time there is no evidence that this species parasitises roots and it is perhaps best to regard it as a semi-parasite or free-living species.

Systematics.

A few remarks are necessary in relation to *Tylenchus dihystera* Cobb, 1893a, as a synonym of this species. Unfortunately Cobb gave no drawing



Anguillulina robusta (de Man).

Fig. 25.—Head end of male specimen, highly magnified, in lateral aspect.
Figs. 26–28.—Three female tails, highly magnified, in lateral aspect.
Figs. 29 & 30.—Two male tails, highly magnified, in lateral aspect. Note the shape and structure of the gubernaculum.

of his species and one must, therefore, rely on the formula and brief description which he gave. From these the four following features indicate, in the writer's opinion, the probable identity of the worm with

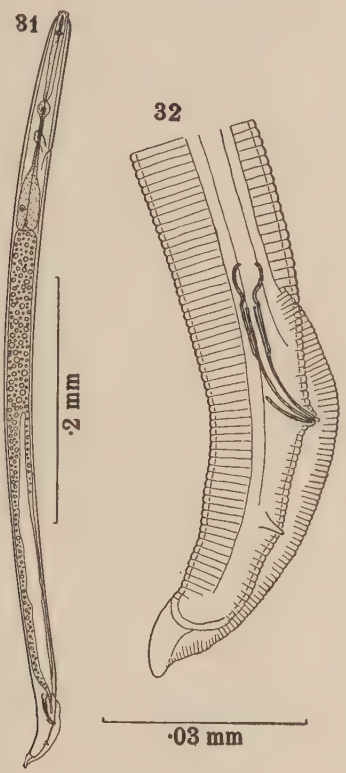
A. robusta. 1. Its length is given as 0·85 mm. This is a little shorter than 0·88 mm., the length of the shortest female specimen found by the writer but it is of the same order and in any case not much stress can be laid on total length as a diagnostic character. 2. The length of the buccal spear is given as 28μ which agrees well with the length found by the writer for this organ in females of *A. robusta*, i.e., 28μ–30μ and is longer than the spear in females of *A. erythrinae* where it is 24μ–25μ long. 3. V, the percentage distance of the vulva from the anterior end, is 57%, i.e., somewhat more forward than in females of *A. erythrinae* where the most anterior value of V found by the writer is 58·3% and nearer to the average position for V in females of *A. robusta*, i.e., 55·05%. 4. In describing the tail, Cobb says :—" the ventral contour of the conoid tail was continuous with that of the belly, there being no bend or curve as on the dorsal side." This is not very precise but at any rate it vaguely fits the shape of the female tail of *A. robusta* in which the ventral side is continuous with the ventral line of the body, the rounding being from the dorsal side. One may possibly infer, also, that the tail exhibited no tendency to form a ventral terminal process as in *A. erythrinae*. Had it done so it would surely have been noted by Cobb.

Steiner has stated, in a review of the writer's book " Plant Parasitic Nematodes and The Diseases They Cause " (*J. Parasit.*, 21 (1), p. 65, 1935) that the nematode figured therein as *A. multicincta* in fig. 96, p. 231, is *A. dihystera* (Cobb). This is an opinion against which may be set the reasons adduced above for considering *A. dihystera* (Cobb) as a synonym of *A. robusta*. Steiner's further statement, in the same review, that *A. multicincta* (Cobb) is a synonym of *A. robusta* (de Man, 1876) is clearly erroneous as the descriptions of these two species given in the present paper abundantly prove.

Anguillulina obtusa (Bastian, 1865) Goodey, 1932.
syn. *Tylenchus obtusus* Bastian, 1865.
Rotylenchus obtusus (Bastian, 1865) Filipjev, 1936.

The account of the female of this species given in the writer's 1932 paper was based on two specimens only. Since then many more females have been obtained in water extractions of turf at this Institute. Females and larvae have also been found within the cortex of the roots of oat seedlings and certain grass species. In addition three adult male worms

have been obtained and from these it is possible to amplify the very meagre description of the male given by Bastian and to present an adequate drawing of the male tail.



Anguillulina obtusa (Bastian).

Fig. 31.—Male under moderate magnification to show general shape and structure.
Fig. 32.—Male tail, highly magnified, in lateral aspect.

Morphology.

Dimensions : *Female*, length, 0·55 mm. to 0·8 mm., α=24–30, β=3·8–5·8, γ=13·5–17·6, V=55·2% to 62·7%. Average values based on 33 specimens, length, 0·627 mm., α=26·8, β=4·3, γ=14·7, V=58·8%.

As these dimensions show, the female exhibits a considerable range in length and it is interesting to note that the longest specimens obtained have practically the same length as that given by Bastian for the female i.e., 0·8 mm.

There is nothing to add to the technical description of the anatomy of the adult female given in the 1932 paper. Females obtained from the cortex of grass roots show one egg in each uterus and from the appearance of such specimens it is clear that each uterus is capable of holding one egg at a time.

Male: length, 0·6 mm. to 0·69 mm., α=24·1–28·6, β=3·4–4, γ=13·4–13·8, spicules, 27·5μ, gubernaculum, 9μ. Average values based on 3 specimens, length, 0·64 mm., α=25·9, β=3·8, γ=13·6.

Head, buccal spear and oesophagus are the same as in the female and call for no special description. Gonad single, outstretched anteriorly and extending about halfway down the intestine. Fig. 32 shows that the tail tapers very slightly to a rather bluntly rounded terminus which is completely enclosed by the bursa. The latter is rather long and arises at about the level of the heads of the spicules and has a crenate edge corresponding to the striations of the bursal wings. On each side of the tail there is a well defined papilla situated post-anally about halfway between the anus and the end of the tail, its tip not reaching the free edge of the bursa. Each spicule is slightly arcuate, the anterior end is rounded when seen in lateral aspect and is cephalated by constriction from the rather narrow shaft which tapers to a point. The gubernaculum is small, simple and slender.

Occurrence.

The species is parasitic in habit. Egg-bearing females and larvae have been found by the writer in the cortex of the roots of the three following grasses dug up at this Institute; *Agrostis stolonifera* L., *Lolium perenne* L. and *Dactylis glomerata* L. As a rule the worms lie stretched out parallel to the long axis of the root and the body may extend through two or three cells the walls of which are perforated by the parasite. Occasionally specimens may be found with the body folded on itself like a letter U. The species thus resembles *A. pratensis* in its parasitic habit and may in fact be very easily confused with the latter species when seen in roots stained with acid fuchsin-lactophenol. Egg-bearing females and the eggs recently laid by them have been found in the roots of all three species of grass named above when dug up and stained in December 1939.

It is also worthy of note that in these stained grass roots, at any rate in those of *Agrostis stolonifera* which is the predominant grass species in the pastures here, *Anguillulina obtusa* appears to be more numerous than

other root invading species. In some roots it is often the only species present. In other cases there may be an occasional representative of *Anguillulina pratensis*, *A. erythrinae* or *A. costata*. It is also commoner in main roots than in small branches though it does occur in these as well. Again, it is only females, eggs and larvae which are found within the root cortex ; the adult male does not appear to invade root tissues. Out of some hundreds of representatives of this species dissected from grass roots during the past winter months, the writer has not discovered a single male worm ; the males described above were obtained by the Baermann funnel technique. One would scarcely be justified in inferring from this apparent scarcity of males that the females are parthenogenitic since spermatization of the female may occur whilst she is free in the soil. In contradistinction to several species of the genus *Anguillulina* in which a particular larval stage serves as the infective stage, the adult female of *A. obtusa* appears to be the usual infective stage. The writer has found several examples of egg-bearing females entering roots ; the head end being buried in the cortex with the rest of the body lying free outside.

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On Wild Birds as Transmitters of Helminth Parasites to Domestic Stock.

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THERE has been considerable speculation as to the importance of wild birds in the transmission of helminth parasites to domestic stock, most of the observations having been directed towards gapeworms. As far back as 1903, Klee had associated an outbreak of gapes in a pheasantry with a near-by rookery where over 50% of the rooks carried gapes during the winter. Lewis was of the opinion that some wild birds, in particular the starling, were the cause of sudden outbreaks of gapeworm disease in districts near Aberystwyth. He says "adult starlings, turkeys and pheasants infected with *Syngamus trachealis* act as a bridge between the period of possible infection of chickens from one year to another, and thus keep up a connected period for the propagation and distribution of *S. trachealis*."

"For example, when there are no chickens for the gapeworms, starlings are generally abundant, and are infected to a high percentage; and when the starlings are about to migrate . . . chickens appear, and may act as hosts for the gapeworm." He comments on the fact that starlings frequent poultry runs in large numbers and will contaminate the soil with eggs.

Taylor did not, however, agree with these conclusions. His attempts to transmit to chickens, gapeworm of starling origin met with but little success in the first generation. He was therefore of the opinion that starlings were not of prime importance in the field.

A study of the literature shows that other helminths have not been considered from this aspect, except for one short paper in Russian which deals with the mechanical carriage of eggs by sparrows. Some experiments have been carried out by the present writer to assess the importance of wild birds as carriers, not only of gapeworm, but also of other parasites. The first results follow.

The problem of gapes has been considered very fully in other papers and will be disposed of briefly here. The conclusions drawn by Taylor and by Lewis both contain some of the truth. Since the demonstration of the importance of the intermediate host in the life-history of gapeworm, it is obvious that starlings, turkeys, etc., may act as carriers but not precisely as Lewis suggested. They contaminate the soil and give rise to some infections by direct development, but only occasionally. But they also contaminate the invertebrates capable of acting as intermediate hosts and thus set up reservoirs of infection for future use. This is a more insidious action for such infections are not at first obvious and later it is difficult to assess the degree of infection and almost impossible to eradicate it. Therefore the birds immediately assume an importance that they did not have before. For the intermediaries remain infected for a long period of time and are continually re-infected as a certain percentage of starlings carry gapeworm throughout the year. The same may be said for the rook and to a less extent for other members of the Corvidae and for some of the gallinaceous birds, which act as hosts for *Syngamus trachea*.

A survey of the helminth parasites of various common wild birds around St. Albans, notably rooks and crows, is proceeding as part of this investigation. The other part consists of putting clean birds on land whose past history is known and of examining the resulting infections after varying periods of time.

It should be made clear here that the only known helminths established on Winches farm among poultry are *Heterakis gallinae, Ascaridia lineata* and *Capillaria longicollis*. Some ducks imported as young adults had infections of *Fasciolaris fimbriaria* but they may have brought this in with them. Other helminths have never been met with in the avian stock here.

For the first experiment, a piece of slightly sloping land, which had not carried birds for at least 6 years, was used and a number of turkeys, a pair of guinea fowls and some chickens were given the run of several pens. The experiment had to be terminated at the end of 6 months owing to an outbreak of tuberculosis, but by that time a guinea fowl and some of the adult turkeys had died of an intense enteritis and haemorrhage due to the invasion of the intestinal mucosa by hundreds of larvae of *Ascaridia sp.* This is particularly interesting as by this time all the birds were adults and it is unusual to find fatal infestation of *Ascaridia sp.* in adult birds.

There were also some adult *A. lineata*, *Heterakis gallinae* and *Capillaria longicollis*. There were no cestodes.

Later on two other plots were used for a similar experiment. Neither had carried birds during the tenancy of the Institute, which dated back for a period of more than 16 years. One had been entirely enclosed by close mesh wire netting for 4 years, thus excluding all wild birds. The other was open to all birds and small mammals, but had only carried sheep and goats. Parasite-free chicken poults were introduced to these plots of land and were subsequently slaughtered at intervals and examined for helminths. The open plot of land proved very attractive to wild birds which collected there in large numbers for feeding purposes. There were often several hundreds of rooks and starlings. Other birds frequented it also but in fewer numbers. There were blackbirds, thrushes, linnets and sparrows in fair numbers and occasional jays, crows, tits and finches and other birds of the countryside. An occasional pair of partridges appear and nest on the farm land though they have never been seen in this pen.

It may be said now that the birds in the enclosed pen never developed any helminths during the next 7 months when the experiment was finished. The others quickly became parasitized. After an interval of one month a bird was killed and showed an infestation consisting of *Capillaria longicollis*, *Heterakis gallinae* and *Trichostrongylus tenuis*. This one was very lightly infected; birds killed later showed the same parasites but in larger numbers. At the end of 7 months, the course of the experiment had to be changed as a severe snow storm broke through the wire netted cage so that the enclosed chickens could wander over wider pastures and the entry of small birds was made possible. It is significant that these hens became infected with *H. gallinae* within 3 weeks of attaining their freedom, so that they were not immune to helminth parasitism.

Later, a batch of day-old incubator-hatched chickens was put under a broody hen in the open cage and allowed free range with the older hens that still remained. These birds were all cockerels.

The first was killed 7 weeks later and contained 7 specimens of *Hymenolepis corvi* and some young *H. gallinae*. The following week another one was killed and this too contained one *H. corvi* and some of the nematodes. Some of the rooks feeding from this ground were shot at this time and between them they showed infections of *H. corvi*, *H. serpentulus*, *Syngamus trachea*, *Porrocaecum ensicaudatum* and *Capillaria ovopunctata*. No

Hymenolepis spp. have ever been recorded from the domestic fowls of this Institute before and we must assume therefore that this species was introduced by the rooks and here found access to the right intermediary, which is as yet unknown.

The remaining birds were killed at monthly intervals. Cestode infestations occurred only in the first two birds. *Capillaria longicollis* were present in small numbers in the fourth bird killed. *Heterakis gallinae* occurred in all the birds and the number gradually increased with each bird. The highest number obtained from one bird was over 500. Most of the worms were young forms. There were rarely more than 200 adults present at a time. *Ascaridia lineata* was recovered from the last three birds killed—not in large numbers : six was the largest number recorded and none seemed to have matured, thus bearing out the observations of Ackert and later of Roberts, that some immunity occurs, whereby the development of the worms in adult chickens is stunted.

The third observation that has a bearing on this subject deals with parasitism in pheasants. A hatching of pheasant poults was released in a small pen and lived there for about a year. At the end of that time they had all either died or had been killed and two of them were found to contain Acanthocephala in appreciable numbers. There was a slight reaction in the intestinal mucosa at the points of attachment. The species involved was *Mediorhynchus micracanthus*, a parasite which normally occurs very commonly in starlings but which is not a parasite of gallinaceous birds.

These experiments are by no means finished but it is interesting to notice the degree and type of infestation of helminths that occurs on land that has not carried birds for some considerable time. It is suggested that such infestations do not lie about for years, waiting dormant until the right sort of definitive host turns up. Though some helminth ova can withstand atmospheric conditions and remain viable for considerable times, yet periods of up to 16 years are very long. Furthermore in the pen which had been enclosed by wire mesh for 4 years, the chickens remained clean of all helminths for many months, which suggests that eggs that had been deposited there in times past had not remained alive. It seems more likely that ova are continually re-introduced by wild birds and perhaps by rabbits or other small mammals which range freely over a wide area. Birds, particularly rooks and starlings and sparrows are

practically the only means of communication that these fowls had with the outside world. The eggs may have been carried mechanically and accidentally among the soil attached to the feet, beaks and hair on such carriers or they may have been picked up with the food and passed unchanged through the gut. Evidence will be published later that wild birds carry ova both on the feet and in the gut and that both methods of carriage are in force in the passage of infections to clean pastures.

Whatever may prove to be the explanation, the fact remains that infections of *Capillaria*, of *Heterakis*, and of *Ascaridia* have appeared on so-called clean land by some means. While Heterakis and Ascaridia infections seem to get banked up and more intense, Capillaria appears and disappears sporadically.

The cestodes, Acanthocephala and *Trichostrongylus tenuis* come into a different category. They are all normal parasites of some of the common visitors and have become established in the chickens or in the pheasant. It is not yet known how important are such parasites of the Corvidae and other wild birds to domestic stock. Probably they have no great significance economically as old birds are probably immune to infection with them. In these experiments, it was only the young chickens that took infections with *H. corvi*. The case of *T. tenuis* may, however, not be so simple as it has recently been observed that domestic birds, when adult as well as when young, can be infected with this parasite and that the parasite can produce lesions and even death in these unusual hosts. Furthermore it passes through its complete life-history within a very short space of time and so re-infection can soon occur.

Other parasites may be carried about and established in new areas by accidental contamination by wild birds, and over a period of time it may be that changes may occur in the helminth fauna of a district and enrichment be brought about.

Post-mortem examination of a large number of rooks and starlings has revealed the presence of a very abundant fauna. Among the nematodes, *Capillaria ovopunctata* and *Porrocaecum ensicaudatum* are of frequent occurrence. *Syngamus trachea* also occurs. The Acanthocephala are represented by *Mediorhynchus micracanthus*. Cestodes are fairly abundant and consist mainly of species of *Hymenolepis*. Trematodes have not been met with at all. Most of these helminths have not yet been recovered from or transmitted to birds of economic importance and may not even be capable of transmission.

SUMMARY.

Evidence is put forward to show that eggs of helminth parasites are being constantly re-introduced to land and transferred from one pasture to another. In this way land which has not previously carried poultry for years may yet carry viable eggs which can, and do infect chickens when they are introduced to such runs. The starling and rook are probably important transmitting agents as these congregate among poultry at feeding times. Rabbits and other small rodents may also be carriers but no evidence for this has been looked for yet. Young poultry stock may also become infected with helminths which are normally parasites of wild birds but such parasites, except one Acanthocephalan, have not yet been recovered from or transmitted to older birds experimentally.

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Studies on *Coenurus glomeratus.*

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Multiceps glomeratus is a parasite of carnivores, the larval stage of which is a coenurus which can develop in various small rodents. The adult is a typical taenioid tapeworm measuring up to 70 cm. long. It can be recognised by the shape, size and number of the hooks which have already been described by Turner & Leiper in 1919.

A dog harbouring *Multiceps glomeratus* was slaughtered and yielded up 25 cestodes. The free segments were collected, together with the last 3 from each worm. They were teased up to liberate the eggs which were then fed in bread and milk to a group of 23 mice, with the object of obtaining the larval stage. The mice were examined frequently but there was no trace of cysts until 10 months after infection when one appeared under the left armpit of a female. This grew from a very small size about 2 mm. diameter to one of 19 mm. diameter in the space of 26 days. The mouse was then killed and on examination of the cyst, 53 scolices, typical of *M. glomeratus*, were found. It was fed to a dog which began to pass eggs and segments 7 weeks later.

During the next two months, 7 more mice showed that they had acquired the infection, most of them having multiple infections—one mouse had as many as 5 cysts, 5 others had 4 and the other developed 2. They were situated just under the skin in various parts of the body; the head, shoulders and back were sites that were often chosen. In all cases it was apparent that the cyst, once noticed, grew rapidly and seemed to become infective in about 6–8 weeks after it had first been seen. The mouse with the 5 cysts was killed 5 weeks after they were first noticed as small growths but they were not mature, for the hooks were not completely developed. Though they were fed to 2 dogs no infections resulted. Most of the other mice were killed later and the cysts fed to 2 dogs and 6 cats. Good infections were obtained in both the dogs but the 5 kittens and 1 adult cat proved refractory to infection. Post-mortem examination after varying periods of time failed to show any traces of tapeworms.

The cat would not seem to be a very successful definitive host for this species.

Most of the infected mice were allowed to live and the whole course of the infection followed up. They have a very short life, for about 6 weeks after reaching maturity they began to subside and disappeared in the course of about 3 weeks. Post-mortem examination at the sites of infection revealed a mass of caseous material in which traces of hooks could still be seen. It was surrounded by a zone of inflammation with round cell infiltration.

Later in the experiments, the suitability of rabbits as intermediaries was investigated, 4 adults being used. Of these 3 did not take the infection but one developed some cysts located as follows :—2 on the back, 1 in the groin and the other under the left armpit. The first 2 appeared simultaneously 11 months after the experimental feedings and the others a month later. Two of these were excised under local anaesthesia and feedings to dogs proved them to be at the infective stage. I am indebted to Mr. J. W. G. Leiper, M.R.C.V.S., who kindly performed these operations for me.

The cysts in the rabbit persist for a longer time than in the mouse. They continued to grow until a very large bulk of parasitic material was present. The rabbit died as a result of these cysts 20 months after it was first infected and the body was found to contain a large number of cysts, which were not, however, strictly comparable with those found in the mouse.

Description of the Cysts.

The cysts from the mouse are very similar to those described by Turner & Leiper. They are roughly spherical in outline, small and at their full development measure from 5·5 mm. to 27·2 mm. in diameter. They were all placed under the skin, between the muscles which they had pushed aside until they came to lie in a deep depression so that only a small portion was visible on the surface. They were thin walled and were surrounded by a thin adventitious cyst, probably of host origin. Each cyst was entire and showed no evidence of proliferation or budding. The scolices were arranged roughly in double rows, radiating from a common centre, rather like *C. serialis* but not occupying the whole of the internal surface of the cyst.

The cysts from the rabbit were very different. Those on the limbs were intramuscular, entire and conformed very closely to those found in the mouse, except that they were larger. But there had also developed a large number of abdominal ones and these were large, proliferating bodies that eventually came to occupy the whole of the abdominal cavity, pushing their way through the viscera and bringing great pressure to bear on the vital organs of the host. At first sight, there were apparently 6 coenuri in the abdominal cavity, each encased in a thick fibrous tissue adventitious cyst, attached to the peritoneum. When these were dissected, however, it was noticed that each adventitious cyst contained more than one coenurus, there being 25 in all. The largest cyst measured 121 mm. long by 76 mm. broad. The cyst walls were all thin, so that the scolices were conspicuous as opaque masses arranged roughly in double rows. They tend to be localised and the whole of the internal surface is not used for the buds. An area of at least $\frac{1}{8}$ of the cyst wall is completely clean of all scolices exactly as in the mouse. Many of the cysts were proliferating and " daughter cysts " were being budded off externally and internally. All stages of development were present and will be described later. The cysts were filled with a clear fluid and were for the most part turgid. Some, however, were quite slack. This fluid has a specific gravity of 1·0098 at 20°C. It was very strongly alkaline ; the pH, measured by the electrometric method, being 8·22. The total solid content was 2·06 per cent., of which 0·46 per cent. was shown to be metallic after ashing. It has not been possible to do a further more detailed analysis. Between the parasitic cyst and the adventitious cyst was a slightly hyaline fluid, yellowish in colour, which will be discussed later.

The buds measure in the neighbourhood of 1mm. long by 400μ broad and carry a typical taenioid scolex with 4 suckers and a double row of hooks. They are full of calcareous granules. The number of buds varies considerably : there were as few as 23 in one cyst from the mouse while another from the same animal had 75 and yet was approximately the same size. One removed from a rabbit carried 502 buds.

These cysts from the rabbit approximate closely with those obtained from the gerbille. The one described by Railliet & Henry had small protuberances on the surface which would seem to be an early stage of proliferation. The dorsal surface and the head, particularly over the eyes in the mouse, were often affected but the armpits and the groin have also been used. In the gerbille, the abdomen seems to be the most

frequently affected region and there the cysts grow to a large size and proliferate. Some subcutaneous cysts however, have also been found in this host. In the rabbit they develop both subcutaneously and intraperitoneally.

The occurrence of multiple infections has been the rule in all these animals.

The time factor is interesting. Development is extremely slow. At least 10 months elapse before any signs of infection appear, after which growth occurs rapidly so that maturity occurs in about a year, in both these experimental animals. In the gerbille, the cysts show prominently in under three months. They have persisted for nine months in a single animal, when it died and the post-mortem showed that the parasites were viable. There was a large proliferating cyst in the abdominal cavity and several subcutaneous ones in the region of the head and thorax.

The ease and speed with which the coenurus develops in the gerbille suggests that this, or some closely related species, is one of the natural intermediate hosts. But other rodents are facultative intermediaries. The gerbille itself does not occur in West Africa from which district the original coenurus was described. A closely related species, *Dipodillus campestris*, is indigenous to this region and may act as a natural vector there. Rabbits, when susceptible, seem to make excellent vectors. The experimental animals used were too few on which to make any definite statements, but it is doubtful if rabbits can be considered as natural vectors, for 25 per cent. is too low a positive result if they were naturally infected. Moreover the development in this animal is too slow.

The effect of the change of host on the development and nature of the parasite makes an interesting observation. The slowing down of the development is very marked in the mouse and the rabbit as is also the speed with which breakdown and decay set in, in the mouse. Yet 7 out of 23 mice, or 30·4% is a very fair percentage of positives for an experimental infection in an abnormal host, particularly as most of them gave rise to multiple infections. The other mice in this group were consistently refractory to infection for in spite of repeated feedings with ripe eggs, they still show no signs of developing cysts.

All stages of development were apparent in the single infected rabbit. Most of them were obviously mature with a large number of developing scolices. There were smaller ones with a few immature scolices : smaller

ones still where the scolices were represented only by masses of calcareous granules in which differentiation had not occurred. Even smaller ones still in which there was no trace yet of scolex development. What may prove to be an early stage of daughter cyst development was apparent in two of the largest cysts. A number of scolices had detached themselves from the germinative membrane and had developed a small vesicle posteriorly while the scolex was beginning to show signs of degeneration. They were of a dusky pink colour and this was associated with the calcareous granules. It bore no relation to haemoglobin. Still attached to the mother cyst wall were other scolices containing pigment. They were beginning to degenerate and to show signs of the development of a posterior vesicle. While all these forms need further histological investigation, it seems as if we have here several stages in the development of endogenous daughter cysts and the process bears a close relationship to what is known to occur in hydatid. Daughter cysts are not unknown in the type of larva known as a coenurus, though they are not common. They have been recorded from *C. serialis* by Railliet. He figures and describes coenuri from the rabbit in which proliferation occurs with daughter cyst formation. A further point of resemblance with hydatid is seen in the presence of fluid between the parasitic and adventitious cysts. Such a fluid tends to occur in hydatid when the cyst has been pierced by surgical interference or has suffered traumatic injury. As will be described later in this paper, one of these cysts had been pierced several times with a needle and fluid withdrawn but only one cyst, lying above the left scapula, had been so treated. Yet this fluid was apparent in several of the cysts.

Host Reactions.

Neither mice nor rabbits seem to be seriously affected by the presence of the coenuri unless a large bulk of parasitic material has developed. For a very long time vitality, movement and reproduction are unimpaired. In degenerating cysts, a caseous mass is present which is gradually absorbed by leucocytic action. Sections of the surrounding tissues showed very heavy infiltration with small round cells—a general reaction to any foreign body—and some eosinophiles, which constantly occur in association with all helminth parasites. A thin host membrane is formed round the parasites in the mouse and a very thick fibrous one in the rabbit. Much adhesion of the abdominal cysts to the wall of the peritoneum was apparent.

The non-toxic nature of the fluid was abundantly demonstrated when the two cysts were removed from the rabbit. The second was taken away 16 days after the first. In both operations the cysts were ruptured and the fluid dispersed. But in neither case was there any reaction in the host. There was no inflammation, no fever and no loss of vitality. Both wounds healed normally and the other cysts were not affected.

Fluid was removed by syringe from one of these cysts and injected into the infected rabbit. The animal received 0·25 cc. fluid intradermally and 5 days later 0·5 cc. intravenously. Similar injections were given to clean rabbits and to some infected with *C. serialis* and *Cysticercus pisiformis*. Those free from *C. glomeratus* gave a negative reaction to all the injections. The glomeratus-infected one gave a positive result to the intradermal test. At the site of injection there appeared a wheal about 2 cm. in diameter. This had pseudopodia and was surrounded by a zone of erythema. The reaction appeared 20 minutes after injection and persisted for more than 3 hours, after which it gradually disappeared. There was no delayed reaction. There was no reaction following injections of saline nor with fluid taken from a *C. serialis* cyst, nor was there any response following the intravenous injection.

At post-mortem examination a number of cysts of *C. pisiformis* were attached to the mesenteries round the stomach showing that these two species have no inhibitory action on each other.

Other Coenuri in Mice and Rabbits.

The most common coenurus in the rabbit is of course *C. serialis*, infections with which cannot be differentiated superficially from those of *C. glomeratus*. However, the fact that in a single animal a positive intradermal test resulted from the use of specific antigen, suggests that a serological method of differentiating them may be worked out. At post-mortem examination the size and nature of the hooks would give a diagnosis.

Coenuri among mice are rare. Joyeux in collaboration with Richet & Schulmann reported the presence of a coenurus not yet associated with an adult tapeworm. He called it *C. radians*. It has not, however, been reported since 1922, when it was first described and cannot therefore be at all common. The size and shape of the hooks would serve as a distinguishing feature after death.

Resistance.

While it is recognised that rabbits and mice are not the true vectors of *M. glomeratus* yet they are facultative vectors. The parasite can proceed with its full development in these animals. The comparatively low percentage of positive infections in a group of mice, coupled with the multiple nature of the infections when they did occur, suggests that the resistance is an individual or familial idiosyncrasy. The fact that breakdown is rapid in mice is a pointer that we must look elsewhere for the true vector.

Some carnivore is almost certainly the true adult host and the dog has proved amenable to infection in the laboratory but the cat has proved resistant.

The presence of a tapeworm of this species already living in a dog confers no immunity on the animal for it is possible to increase the infection by feeding further scolices.

Summary.

1. Infections with *Coenurus glomeratus*, which has been reported from West Africa, have been accomplished successfully in the mouse, in the rabbit and gerbille.

2. Such coenuri produced infections of adult cestodes in the dog but not in the cat.

3. The coenurus is described from each intermediate host.

4. The course of the infection in the rodents has been followed and certain differences, depending on the nature of the intermediary, have been noticed.

5. Positive intradermal tests have resulted in the rabbit from the use of specific antigen.

6. Antibodies in the rabbit would not react with antigens from *C. serialis*, *C. pisiformis* or with physiological saline.

7. Antigen from the rabbit would not react with antibodies in animals infected with *C. serialis*.

8. Dogs already carrying an infection of *M. glomeratus* can be further infected by feeding more coenuri.

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Haematological Studies on the Gut Contents of Certain Nematode and Trematode Parasites.

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INTRODUCTION.

THOUGH many species of parasites are said to suck blood, the actual detection of blood or its resorption products in the intestines of the parasites has seldom been carried out. Looss (1905) and Fauré-Fremiet (1912) studied certain reddish-brown, weakly bi-refringent sphaerocrystals occurring in the intestines of *Ascaris, Strongylus, Ancylostoma* and *Trichuris.* These workers came to the conclusion that these particles which were insoluble in water, sodium hydroxide, acetic acid, ethyl alcohol and xylol and were not affected by saliva, gastric or pancreatic enzymes, were probably the result of haemoglobin resorption. Later Liévre (1934) studied the intestines of *Ascaris lumbricoides* and *Parascaris equorum* spectroscopically but failed to detect haemoglobin. He was successful, however, in finding haemoglobin in the intestines of 75% of the specimens of *Toxocara canis* he examined by this method. Ashford Igaravidez (1911) examined Ancylostomes and found blood to be absent from the intestines of many specimens. However, Wells (1931) and Nishi (1933) actually observed *Ancylostoma caninum* sucking blood and stated that these worms appeared to need a copious supply of arterial blood. Red blood corpuscles and eosinophiles have been found in the intestine of *Metastrongylus elongatus* from the pig (Hung, 1926). Hoeppli (1927) never found red blood corpuscles in the intestine of *Ascaris,* though it always gave a benzidine reaction.

The evidence as to the blood sucking habits of parasitic worms thus seems somewhat contradictory and the present investigation was undertaken to discover whether more delicate methods of determining the presence of haemoglobin could give more definite information. Attempts were made to find, if possible, the nature of the changes undergone by the ingested haemoglobin in the worms' gut. From the pathological point

of view the amount of blood taken in by the parasites is of great importance and where possible the quantities of haemoglobin and its products present have been estimated.

Spectroscopic Technique.

The necessity for detecting very small quantities of haemoglobin suggested that spectroscopic methods would prove satisfactory. For qualitative examination a Hartridge reversion microspectrometer, calibrated against the chief Fraunhofer lines and the arc spectra of Li, Ba, Na, Cr and Ca was used. The calibration was frequently tested by means of the chief neon lines. The strength and position of the light used in conjunction with the spectroscope was standardised. The microscope on which the microspectrometer was mounted was fixed in position. Small glass cells holding a column of fluid 0·53 mms. in length were used to hold the solutions under examination.

Quantitive measurements were obtained by the use of an instrument somewhat similar to that described by Harrison (1938). This consisted of a small hand direct vision spectroscope mounted on a solid glass rod which could slide up and down in a glass cylinder. The solution to be examined was placed in the cylinder and the end of the glass rod pushed down into it. Care was taken to ensure that no air bubbles were imprisoned under the end of the rod. Light from an ordinary bulb was passed through the solution under examination, up the glass rod and through the spectroscope. By raising or lowering the spectroscope the length of the column of fluid through which the light passed could be varied. Mounting the spectroscope with the solid glass rod attached in the barrel of a microscope from which the nosepiece holding the objectives had been removed allowed the spectroscope to be raised or lowered with precision. Using solutions of globin-haemochromogen of known concentration (estimated as the equivalent quantity of haemoglobin by Wong's (1928) method) the lengths of the columns of fluid at which the strong absorption band became extinguished were measured. The lengths of the columns were then plotted against concentration of globin-haemochromogen and by reference to the resulting curve the concentration of haemochromogen in unknown solutions could be estimated.

Microscopic Examination of the Gut Contents of *Strongylus* spp.

Microscopic examination gave little evidence as to the nature of the intestinal contents. Smears were made from various sections of the gut.

At the anterior end the contents were fluid and bulky with very little apparent structure. Staining with haematoxylin revealed small bodies which may have been partially digested blood corpuscles and epithelial cells. The posterior sections contained a larger number of particles. Most of them were small brownish spherical granules about 4μ in diameter. These failed to dissolve in potassium hydroxide though spectroscopic examination after the addition of reducing agent showed that some haemoglobin product had gone into solution.

Examination of sections of the gut cut at intervals along its length showed that the small brown granules were situated in the gut wall just inside the bacillary layer. The lumen of the intestine in the posterior region contained very small black particles which dissolved in potassium hydroxide and it is thought that these particles represented the haemoglobin product discussed in the paper.

Spectroscopic Examination of the Gut Contents.

(1) *Strongylus edentatus* : The intestines and their contents taken from several specimens were incinerated in a crucible and the ash dissolved in dilute hydrochloric acid. The resulting solution gave positive tests for iron with potassium ferrocyanide and potassium thiocyanate. The presence of iron gave strength to the surmise that haematin was present. Accordingly attempts were made to dissolve the intestine and its contents in pyridene and ammonium sulphide. Solution took place only with difficulty and the spectroscope revealed no bands except on one occasion when a solution in the latter solvent showed haemochromogen bands. Boiling with 10% potassium hydroxide, however, gave a solution which, on the addition of sodium hydrosulphite showed very strong haemochromogen bands. Occasionally unchanged oxyhaemoglobin could be detected in the anterior section of the intestine.

In obtaining quantitive results the following routine was observed. A known number of female *S. edentatus* were taken from a newly slaughtered horse, washed in physiological saline, dried by rolling gently on filter paper and weighed. The worms were then replaced in saline from which they were taken one by one and the intestine dissected out. This was done by cutting off the head and tail and then drawing the intestine free by gentle traction at the anterior end. The intestines were washed rapidly in distilled water to remove the body fluid on their outer surfaces and then placed in small test tubes containing 0·5 mls. of water.

Here the intestines were broken up and the whole contents well stirred and centrifuged. The supernatant fluid was then pipetted off and examined in the Harrison spectroscope. Only on one occasion were the bands of oxyhaemoglobin detected in this solution. It is evident, therefore, that the digestion of the ingested haemoglobin takes place rapidly.

After extraction with water the material remaining in the tube was heated with 0·5 mls. of 10% potassium hydroxide. The supernatant fluid, a brownish-green solution, was pipetted off after centrifuging. Spectroscopic examination of the solution revealed no bands but on the addition of a reducing agent (a little solid sodium hydrosulphite) the solution became brownish-red and the bands of a haemochromogen appeared. Three samples of 0·05 mls. of this solution were diluted with known amounts of water and examined in the Harrison spectroscope by means of which the amounts of haemochromogen present were estimated as the equivalent quantity of haemoglobin. An average of the three results was taken and from this figure the amount of haemoglobin necessary to form the haemochromogen extracted from the intestines of the worms was found.

Table I shows the figures obtained in estimating the haemochromogen in one lot of parasites' intestines.

Table I.

Sample.	Dilution.	Average length of column of haemochromogen.	Concentration of Hb. in mgs. per ml.	Amount of Hb. in the 0·5 mls. KOH.
I ...	0·05 mls. soln. 0·5 mls. H_2O.	0·45 cms.	1·76	9·6 mgs.
II ...	0·05 mls. soln. 0·75 mls. H_2O.	0·70 cms.	1·38	11·0 mgs.
III ...	0·05 mls. soln. 1·25 mls. H_2O.	1·30 cms.	0·75	10·1 mgs.

Altogether five lots of *S. edentatus* were examined. The first lot was examined qualitatively only but in the other cases the quantity of the haemoglobin product was estimated as the equivalent amount of haemoglobin. In all five lots a haemoglobin product was present. Table II shows the results obtained.

TABLE II.

Average worm weight.	Average amount of Hb. per worm.	Hb. in worm gut as as % of total worm weight.
75·4 mgs.	1·02 mgs.	1·35
67·6 mgs.	0·48 mgs.	0·71
72·5 mgs.	1·13 mgs.	1·56
67·5 mgs.	0·49 mgs.	0·73

(2) *Strongylus vulgaris*: Three lots were investigated. In two cases the haemoglobin products were estimated quantitatively in a manner similar to that used in the examination of *S. edentatus*. In every case the treatment revealed the bands of globin-haemochromogen. Table III shows the results obtained.

TABLE III.

Average worm weight.	Average amount of Hb. per worm.	Hb. in worm gut as % of total worm weight.
13·7 mgs.	0·11 mgs.	0·80
11·2 mgs.	0·02 mgs.	0·18

Fourth stage larvae of *S. vulgaris* in various stages of development were taken from aneurisms in the ileo-caeco-colic artery of the horse and were found to contain a black pigment in the intestine. Small patches of brown material were also present. The solution of the intestine and its contents in KOH, on the addition of sodium hydrosulphite showed the bands of globin-haemochromogen. It appears, therefore, that immature *S. vulgaris* are capable of digesting blood and the apparent scarcity of other material in the intestine suggests that blood may form a large proportion of the diet of these worms.

(3) Ascaridae: About twenty specimens of *Ascaris lumbricoides* (pig strain) were examined. Of these, only two parasites were found to have haemoglobin products in their intestines. Unless this species is an intermittent blood sucker in which haemoglobin digestion is rapid and complete it may be considered that the presence of blood in its intestine is due to accidental feeding only.

The few specimens of *Parascaris equorum* examined did not have any haemoglobin product in the intestine.

An investigation of *Toxocara canis* and *T. mystax* revealed that a haemoglobin product could be extracted from the intestine. The occurrence of this substance was slightly more frequent than as quoted by Liévre (1934) but as only a limited number of specimens were available for examination this assumption may not be valid. In several cases the haemoglobin product was estimated by the same method as that used in the investigation of *Strongylus* spp. Table IV summarises the results obtained. The first two cases in the table apply to *Toxocara canis* the others apply to *T. mystax*. It will be noted that two specimens of the latter species though small and immature were blood suckers.

Table IV.

Weight of worm.	Amount of Hb. per worm.	Hb. in worm gut as % of total worm weight.
0·1180 grms.	0·17 mgs.	0·14
0·0993 grms.	0·13 mgs.	0·13
0·1572 grms.	0·12 mgs.	0·08
0·0480 grms.	0·06 mgs.	0·12
0·0416 grms.	0·05 mgs.	0·12

(4) *Oxyuris equi* was found to have a comparatively large intestine distended with a bluish-grey mixture. This mixture was to some extent soluble in dilute hydrochloric acid and the resulting solution gave positive tests for iron with potassium thiocyanate. Some of the intestinal contents was then heated with 10% potassium hydroxide. Very little dissolved and the solution after the addition of sodium hydrosulphite failed to show any absorption bands when examined spectroscopically.

(5) The intestines of *Syngamus trachea* were found to contain a black pigment, which, though not plentiful, seemed to form almost all the ingesta. Before treating the intestines with potassium hydroxide it was necessary to wash them well with water for the body fluid of this parasite contains haemoglobin. No haemoglobin was extracted from the intestines with water but potassium hydroxide extracted a haemoglobin

product which, after the addition of reducing agent, could be detected spectrographically.

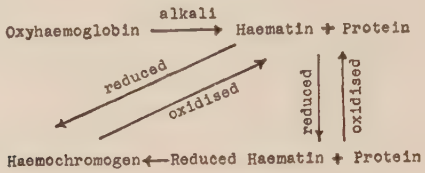
(6) All the specimens of *Schistosoma mattheei* (from a goat) and *S. mansoni* (from a monkey) used had the intestine clearly outlined by a black pigment. In each case about ten whole worms were taken, washed and broken up in water. No bands could be detected by the spectroscopic examination of the water. Potassium hydroxide, however, extracted the black pigment and the solution when reduced showed the bands of globin-haemochromogen.

Discussion.

Of the parasites examined, *Strongylus edentatus*, *S. vulgaris*, *Schistosoma mattheei*, *S. mansoni* and *Syngamus trachea* were probably all blood suckers. It is possible, of course, that the haemoglobin product found in the intestines of the parasites was not obtained from the hosts' blood for the worms themselves may excrete some haemoglobin product in their intestines. However, it would seem unlikely that such large molecules would pass through the intestinal walls. Also the amounts of haemochromogen estimated seem somewhat large for the excretory product of such small organisms. Experiments (Rogers, unpublished) have shown that the body fluid of *S. edentatus* contains on an average about 3·3 mgs. of haemoglobin. This quantity is only about three times the amount required to give the haemochromogen estimated to be present in the intestine. Thus, even if it was possible for very large molecules to pass through the gut wall it seems improbable that such a large proportion of the parasites' functional haemoglobin should be excreted. It may be considered, therefore, that the haemoglobin product found in the intestine was obtained from ingested haemoglobin taken from the host.

The ingested blood is changed profoundly in its passage along the worms' intestines in all the parasites examined except *Oxyuris equi*. Yamada & Kazuo (1934) concluded that neither red blood corpuscles nor leucocytes were digested in *Ancylostoma duodenale*. Hung (1926) stated that the erythrocytes and eosinophiles in the intestine of *Metastrongylus elongatus* were more or less unchanged. Indications that the hosts' blood in the intestines of *Ascaris* is digested are given by Hoeppli (1927).

The following scheme (after Keilin, 1926) shows the relationships between oxyhaemoglobin and allied substances.



Of these compounds oxyhaemoglobin is soluble in water giving marked absorption spectra, whereas haematin is insoluble in water but soluble in ammonia solution and potassium hydroxide. If, in the latter case, ammonia solution is the solvent used, ammonia-haemochromogen is formed, but if potassium hydroxide is used globin-haemochromogen results (Barcroft, 1928). These substances give very similar absorption spectra when reduced.

The above relationships indicate that haematin was the substance extracted from the worms' intestines with potassium hydroxide or ammonia solution. The parasites were therefore breaking down the ingested oxyhaemoglobin into haematin and protein. The fact that the anterior section of the intestine in *Strongylus edentatus* was found to be bulky and contained a somewhat fluid material which in the posterior region seemed to be drier and adhered to the intestinal wall suggests that the protein may have been absorbed in the anterior and middle sections of the gut. Since the haematin was found even in the extreme posterior end of the gut it seems unlikely that it was being absorbed by the parasites.

It seems likely (Hoeppli, 1927 and Rogers, unpublished) that the tissue of the hosts' intestinal wall must form a large part of the ingesta of *S. edentatus* and it is surprising that they should also take in enough blood to give haemoglobin amounting to 1·56% of their total weight. Whether this blood is necessary to fulfil a definite physiological need or is merely accidentally engulfed is doubtful. As the blood is digested and the protein may be absorbed it appears that the former conclusion is correct.

From the point of view of pathology the amount of blood taken from the host by *Strongylus* spp. is small. On the assumption that horse blood contains 12·4 grms. of haemoglobin per 100 mls. (Kuhl, 1919) results (see tables II and III) show that *S. edentatus* takes from 0·0009 to 0·0002 mls. of hosts' blood to give the intestine its normal contents. Again, compared with *Ancylostoma caninum* this is a small amount for Nishi (1933) demonstrated that a single specimen of this species could extract 0·7 mls. of blood from the host in 24 hours. In spite of the fact that Nishi's figures include an amount of 0·216 mls. per day which was not actually ingested by the worms it appears that the species of *Strongylus* studied are of little importance in causing loss of blood unless large numbers of parasites are present.

Toxocara spp. evidently frequently ingest and partially digest the hosts' blood. Considering dogs' blood to contain 13·01 grms. of haemoglobin per 100 mls. the figures of table IV indicate that *Toxocara canis* must ingest up to 0·0001 mls. of hosts' blood in order to obtain the haematin in the amounts found. Since *T. mystax* probably takes amounts of hosts' blood similar to *T. canis* both these species may be considered to be of little importance as blood suckers.

The trematodes studied were parasites in the blood of the host and it is interesting to note that Brown (1911) found that haematin was also a result of the metabolism of the malarial parasites living in the blood.

Summary.

1. The examination of *Strongylus edentatus* and *S. vulgaris* revealed that these parasites ingest the hosts' blood and that haematin is the result of the digestion of haemoglobin. The amounts of blood necessary to form the quantities recorded as being present in the parasites' intestines have been calculated.

2. Evidence has been presented to show that the hosts' blood probably forms a large proportion of the diet of fourth stage *S. vulgaris* larvae.

3. A small proportion of the specimens of *Ascaris lumbricoides* examined contained haematin in the intestine.

4. The intestines of most of the specimens of *Toxocara mystax* and *T. canis* investigated contained haematin. Figures are given to show the amounts found. Even immature forms of the former species were found to be blood suckers.

5. *Syngamus trachea* was found to have haematin in its intestine but no trace of blood pigments could be found in *Oxyuris equi*.

6. Results indicate that the black pigment present in the intestines of *Schistosoma mattheei* and *S. mansoni* was probably haematin formed by the digestion of the hosts' haemoglobin.

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On the Identification of Strains of *Heterodera schachtii.*

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The problem of the identification of the various biological strains of *Heterodera schachtii* has a practical bearing on the control of those strains which are harmful to agricultural crops. It is easy enough to establish the presence of eelworm cysts in a soil, but unless their hosts are known, it is impossible to plan a crop rotation which will not include plants on which the parasite will be able to multiply. If such hosts are cultivated frequently there is a great danger of the eelworm increasing to such proportions as to cause serious damage to crops. Once this has happened it is extremely difficult to eradicate the parasite or even to reduce its numbers. The presence of eelworm on a crop is usually not detected until it is causing damage, and by then it is firmly established in the soil. It is therefore of great importance to attempt to prevent the increase of harmful strains of *H. schachtii* before the soil has become heavily infected.

It is well known that there are two types of *Heterodera* cyst which are easily distinguished by both macroscopic and microscopic characters. The lemon-shaped type, such as the oat, beet and pea strains, has a well marked posterior knob bearing the vulva, and the minute punctation-like marks on the cyst wall are irregularly distributed. The rounded type has no posterior protuberance, and the punctate marks are arranged in rows running round the cyst. This latter type includes only the strain attacking potatoes and tomatoes and the strain, probably identical with the species *H. punctata* Thorne, which has been found on *Agrostis stolonifera* (Franklin 1938). The latter may be distinguished from the potato strain by the fact that the anal and vulval apertures are both readily seen if a cyst which is in good condition is dissected under a low power of the microscope. In the potato strain, on the other hand, the anus is minute in comparison with the vulva, and is not very easily observed. A lemon-shaped cyst, however, may belong to one of several different strains. The beet, oat and pea strains are of this type, and lemon-shaped cysts have

been found on the roots of *Myosotis*, carnation, clover, dock and other cultivated and wild hosts. The ability to distinguish these strains from one another would be of great value in preventing the increase of those which are harmful to agricultural crops.

Methods of Identifying Strains.

The final indication of the strain of a *Heterodera* population is, of course, its ability to attack and reproduce on certain host plants. In order to test this a fairly large number of cysts is necessary. They should be mixed with sterilised soil in which the plants to be tested as possible hosts are grown. It is important that there should be no doubt as to the original host of the cysts being tested, as the inclusion of a few cysts of another strain may give false results. After two or three months' growth the roots of the plants being tested are examined for cysts. This method, though it is the fundamental test in the determination of the infective powers of cysts of unknown strains of *H. schachtii*, is slow and requires large numbers of cysts.

When only small numbers of cysts are available, and information as to their identity is required as quickly as possible, certain laboratory tests can be made which may indicate the strain. One method is to attempt to stimulate the larvae to hatch out of the cysts by the introduction into the water in which they are kept of growing roots of possible host plants, in other words, of the "root excretions" of these plants. Schmidt (1930) used this method to differentiate cysts of the oat from the beet strain. He shows that water in which the roots of growing beet or rape seedlings have been soaked will cause larvae to hatch from cysts of the beet strain, but not from those of the oat strain.

The size of the larvae is an indication in some cases of the identity of a cyst population. Schmidt was able to distinguish oat from beet nematodes by the size of the larvae obtained from the cysts, and proposed the adoption of a *major* and *minor* subspecies of *H. schachtii* on this character.

The above mentioned methods have been used in an attempt to find a means of differentiating several strains of *H. schachtii*.

Infection Experiments.

In order to reduce the time necessary for carrying out an infection experiment, preliminary tests may be made using larvae of the eelworm

to be tested, instead of cysts, and examining the roots of the suspected host by staining them after two or three weeks' growth and searching for entered larvae, instead of waiting for the development of mature cysts. If negative results are obtained in these tests, it is unlikely that the plant will be parasitised by that particular strain of eelworm, but if the results are positive, further tests will be necessary to find out whether mature eelworms will develop from infection by cysts of the original strain.

A convenient medium for staining and clearing roots in order to find out if they are infected is lactophenol coloured with acid fuchsin, in which the roots are boiled for two or three minutes.

(*a*) Potato Strain.

Many infection tests have been carried out to determine what plants the potato strain of *H. schachtii* will attack. It is well known that tomatoes may be seriously damaged if grown in " potato-sick " land. To make certain that it is really the potato strain which parasitises this plant, tomato seedlings were grown in sterilised soil to which was added cysts which had been picked off potato roots. After a month's growth the tomatoes were showing signs of severe sickness, and examination of the roots showed them to be heavily infected by *Heterodera*. No other cultivated host has been found, on experiment, to be susceptible, but two wild species of *Solanum* have become infected when grown in " potato-sick " soil. Two plants of *S. Dulcamara* (bittersweet) when examined after ten months' growth in the infected soil each had 10–15 round cysts on the roots, and *S. utile*, a South American tuber forming plant, readily became infected. Morgan (1925) also found that *S. Dulcamara* became infected when grown in soil infected with the potato eelworm. *S. Capsicastrum*, a plant grown fairly extensively by nurserymen, bore no cysts after several months' growth in infected soil, but only one plant was tested. A native solanaceous plant, *Atropa Belladonna*, the deadly nightshade, was shown to be susceptible. When examined in October, after 4 months' growth in infected soil, no cysts could be seen on the 6 plants being tested, but when the roots of 2 of them were stained, it was found that both contained nematodes. Each had several female *Heterodera*, one of which, though small, was obviously of the round type, and contained embryonated eggs.

(*b*) *Heterodera punctata*.

Numerous tests were carried out with cysts of this type, found on the roots of *Agrostis stolonifera*, as it was desired to find out if this eelworm was likely to be a menace to cultivated crops grown on ploughed up pasture-land. Since these cysts are frequently found in pasture soil their host range is of great interest. Fairly large numbers of the cysts were obtained by flotation from air-dried pasture soil, care being taken to remove the lemon-shaped cysts which were also present in the soil.

The most thorough tests were made with potatoes, as it was thought that the potato strain might have originated from this strain, since it resembles this more than any other. Six potatoes were grown in separate plant pots. One pot contained 600 gms. of sterilised soil, with which were mixed 600 of the *Agrostis* strain cysts, while the other 5 were small pots containing 150 gms. of soil and 100 cysts each. All the plants were examined in June, after about 3 months' growth, but no cysts were found on the roots. The roots were then stained, but still no signs of infection could be discovered.

Tomatoes were also grown with the *H. punctata* cysts during the months of August and September, but they too remained free from infection. Oats and wheat were also uninfected by these cysts. As it was on wheat that Thorne originally found *H. punctata*, these results were unexpected.

Negative results were also obtained when various grasses and weeds were grown with *H. punctata* cysts. Amongst the grasses was *Agrostis alba* (syn. *A. stolonifera*) ; this was grown on several occasions, in some cases for over a year, and in others seedlings were removed from the infected soil after various periods of growth and the roots were stained and examined for larvae, but none was found.

The probable explanation of some at least of these negative results has recently presented itself. Some cysts which had been removed from pasture soil and kept in the laboratory during the winter were soaked in water for several days and then dissected under the microscope. Some were of the lemon type, but most were ovoid. The embryos in the former appeared quite healthy, but in the latter they were in all cases dead and appeared to have become dried and shrivelled. These cysts therefore seem to be very much more susceptible to drying than all the other known strains. This is an additional character distinguishing *H. punctata* from the various strains of *H. schachtii*. Cysts removed from wet soil obtained

in early spring from around the roots of *Agrostis* plants contained living embryonated eggs, and larvae which could be seen, through the cyst wall, moving about inside the cyst. On exposure for a few hours to the dry air of the laboratory the cysts soon became shrivelled and dry. Such shrivelling occurs to some extent in newly formed cysts of other strains, but older cysts removed from soil usually resist drying and retain their shape. The negative results of the infection experiments with *H. punctata* are therefore probably, in some cases at least, due to the lack of viability of the cysts with which the soil was infected, since they had been kept in the laboratory for some weeks before use.

(*c*) Clover Strain.

The lemon-shaped cysts which occur frequently in pasture soil in addition to the rounded type were found to be capable of parasitising both *Trifolium repens* and *T. pratense* (Franklin 1939). Various plants have been grown in soil infected with cysts of this strain in order to find out what other plants would be attacked. Amongst others, oats, sugar beet, mangolds, field beans, green peas, tares, white mustard, dandelion, nettle, field mint (*Mentha arvensis*), forget-me-not (*Myosotis sylvatica*), *Agrostis alba* and *Poa trivialis* were tested. None of these was infected, though white and red clover grown at the same time bore numerous cysts. The green peas were tested on four occasions, on one of which the roots of four plants were stained after $7\frac{1}{2}$ weeks' growth, but no infection had taken place. These experiments, therefore, seem to indicate that the clover strain does not attack those agricultural crops which are commonly damaged by this eelworm, though further trials are necessary to confirm this.

(*d*) *Myosotis* Strain.

Through the kindness of Mr. W. E. H. Hodson, a small quantity of soil was obtained which was heavily infected with a strain of *H. schachtii* which was causing great damage to *Myosotis* cultivated in greenhouses. The cysts were smallish and lemon-shaped, and from the history of the case it was thought that they might have come from pasture soil and therefore had perhaps been parasites of wild clovers. A plant pot containing some of the soil was sown with *Trifolium pratense*, but after 9 months' growth no infection could be found on the clover roots. Four other pots of the soil were sown with *T. repens*: these were examined after 11 months and no infection was found on 3 of them, but there were

4 cysts on the roots of the plants in the fourth pot. Oats and several weeds including a species of *Poa*, a sowthistle and chickweed were also uninfected, while *Myosotis sylvatica* grown at the same time became heavily parasitised. Further trials will be necessary, using cysts picked off *Myosotis* roots, to determine whether this strain really attacks *T. repens*, or whether this was an accidental infection due to the presence of one or two stray clover strain cysts. In view of the fact that on two occasions *Myosotis* was grown in soil infected with clover cysts, and in neither case did it become infected, it appears improbable that the *Myosotis* and clover strains are identical.

(*e*) Carnation Strain.

A further strain of *H. schachtii* of which a few cysts were available for experiment was one which had been parasitising carnations in the South of France. This material was kindly given me by Professor R. T. Leiper, Director of the Imperial Bureau of Agricultural Parasitology. A few of the cysts were mixed with sterilised soil and a plant of Sweet William was grown, as it was thought that a plant related to the original host would be the most likely to be attacked. However, when the roots were examined in July, a year after it was planted, no infection could be found. At the same time only one carnation of four grown for the same length of time in similarly infected soil bore cysts on the roots. Three or four pale yellow cysts were found on the roots which were exposed when the soil was turned out of the plant pot. The following October, when the plants were re-examined, this one had over 50 cysts on the outer roots, and two of the other plants had between 5 and 10 cysts each.

(*f*) Dock Strain.

In July 1939, a small dock plant growing as a weed in a pot of local soil to which no eelworm cysts had been added was found to bear one or two cysts on the roots. This prompted an examination of the roots of dock plants growing at the place from which the soil had been taken, and a plant of *Rumex crispus*, L., was found to be infected. The few cysts found on the docks were of the lemon type and had a well marked sub-crystalline layer. Five of them were mixed with clean soil in a small plant pot and *Trifolium repens* was sown. This was allowed to grow from July 1939 till April 1940, when a careful examination was made of the roots exposed when the soil mass was turned out of the pot. No cysts or white females could be seen. A few larvae which hatched out of the

cysts found on the dock plants were sprinkled in water on to pots of seedling sugar beet and white mustard plants. The roots of the plants were stained one month later, but no larvae were found in them.

(*g*) Sea Maram Grass Strain.

Another strain of eelworm which has been found occurring naturally is that which attacks sea maram grass, *Ammophila arundinacea* Host. Cysts from this host are lemon-shaped and rather large. They have been fully described by Triffitt (1929). Very few cysts were available, but larvae emerged from some of them which were kept in plain water in the laboratory. Twenty-five of these larvae were sprinkled in water on to a growing oat seedling, and after about 9 weeks the roots of the plant were stained and examined for nematodes, but none could be found. A small potato plant and a sugar beet seedling were similarly treated, but also with negative results.

A very great proportion of these infection experiments have given negative results. They are, however, of some value in indicating what plants are likely to be free from eelworm attack, but must, of course, be repeated several times before they can be accepted with any degree of certainty. If the results are confirmed it appears that there is a fairly large number of strains of *H. schachtii*, each having a rather narrow host range.

Stimulation Tests.

The various strains of *H. schachtii* behave rather differently as regards the hatching of their larvae from the cysts. Schmidt showed that the oat and the beet strains differed in that the larvae of the former would not hatch in the laboratory except during the spring and early summer, while those of the latter could be made to hatch at any time of the year. The beet strain larvae showed marked stimulation (see also Rensch), indicated by large increases in the numbers of larvae appearing when the cysts were placed in water in which beet seedlings had been immersed for some hours, as compared with the small numbers appearing when they were in plain water. Oat nematodes, however, showed very little stimulation in the presence of oat root excretion, even during the period of maximum hatching in the spring. At other times of the year they could not be induced to hatch even by this means.

In February and March 1940, an experiment was carried out to test the effect of oat root excretion on the hatching of oat strain larvae. In

each of 10 watch-glasses were placed 20 soaked cysts of the oat strain. To five of them was added distilled water, and to the other five water in which the roots of growing oat seedlings had been soaked for 1½ hours. At weekly intervals the hatched larvae were counted and removed, and fresh liquid was substituted for the old. At the end of three weeks the mean number of larvae hatched from 20 cysts in distilled water was 131·6 ± 17·72, and in oat root excretion 122·8 ± 18·64. In this case there was therefore no stimulation due to the influence of growing oat plants.

A similar experiment was carried out using pea strain cysts and pea root excretion. The latter was produced by placing a germinated pea for one hour in 2 c.c. of water in each of five watch-glasses containing 20 cysts. Five other vessels containing plain water were also set up. Very few larvae hatched in any of the watch-glasses in this experiment. At the end of three weeks, from five replications with 20 cysts each, the mean number of larvae hatched was 7·6 ± 0·5099 in water, and 23·8 ± 9·531 in pea root excretion. These means are not significantly different. The experiment was made at the end of February and beginning of March, when one would expect hatching to take place fairly readily, and it is therefore surprising how few larvae did hatch. The cysts all appeared to be well filled with embryonated eggs. It is possible that the period of greatest hatching of this strain is later in the year.

A more satisfactory strain to use for hatching experiments is the potato strain. It has been shown that only in the presence of some stimulating substance will the larvae of this strain hatch in appreciable numbers in the laboratory. Certain chemicals have been shown by various workers to cause hatching, but potato root excretion is more often used for this purpose.

It was suggested by Professor Leiper that excretions from other plants, in particular from tomatoes, might cause potato strain larvae to hatch. A number of plants was therefore tested, including several species of *Solanum*. In all cases the root excretion was produced by placing three root tips from the plant being tested for one hour in one c.c. of water in which 100 cysts were placed for hatching. The hatched larvae were counted and removed from the dishes at weekly intervals, and fresh water and root excretions were substituted for the old. The experiment was carried on for four weeks during October, and there were five replications for each root excretion. The mean numbers of larvae hatched were as follows:—

	Mean	Standard error	Variation Coefficient
Water	103·2	18·27	39·6%
Solanum Capsicastrum root excretion ...	877	251·8	64·21%
S. tuberosum (potato) ,, ,, ...	710·8	208·5	65·6%
S. nigrum (black nightshade) ,, ...	597·2	102·4	37·97%
S. Dulcamara (bittersweet) ,, ...	585·2	105·2	40·21%
Lycopersicum esculentum (tomato),, ...	377·4	38·09	22·57%
S. utile ... ... ,, ...	192	25·95	30·23%
Oat ... ... ,, ...	86·4	9·39	24·32%
Maize ... ... ,, ...	80·4	8·66	24·07%
Poa trivialis ... ... ,, ...	67·8	6·41	20·89%
Agrostis alba ... ... ,, ...	66	5·26	17·83%

A trial was also made with the root excretions of *Atropa Belladonna*. The root excretion was produced in the same way by placing several tips of the growing roots in the water in which the cysts were placed for hatching. The experiment was carried out in July, and lasted for three weeks. In that time from 100 cysts in potato root excretion, in five replications, a mean of 2,916·6±141·6 larvae hatched, while in *A. Belladonna* root excretion 999·2±151·3 larvae hatched. The variation coefficient was 10·86% in the former and 33·85% in the latter. Although there is unfortunately no comparison with hatching at the same time in plain water, and many fewer larvae hatched in the *Atropa* root excretion than in that of potato roots, a certain degree of stimulation appears to have been brought about by the introduction of the plant roots into the hatching water. The results of the experiments with potato strain cysts are exceedingly variable, as shown by the values of the variation coefficients, but it seems probable that other solanaceous plants besides the potato cause some stimulation, though there is too much variation for a direct comparison to be made of the effects of the different root excretions.

Attempts were made to stimulate larvae to hatch from *H. punctata* cysts by means of root excretions of *Agrostis alba*, and also with leachings from a small piece of turf taken from the pasture where the cysts were obtained. No larvae could be induced to hatch, though attempts were made throughout the spring and summer. A few larvae hatched in March from cysts removed from damp soil and placed immediately in water. It is probable that the cysts used in the first experiments had been allowed to become too dry, and the embryos were dead, as their susceptibility to drying was not known at the time of the experiments.

Cysts of the clover strain which were kept in water which was leached through a plant pot in which *T. repens* was growing also failed to produce appreciable numbers of larvae, though tested both in early spring and summer. Cysts at all stages of development have been found on clover plants, grown in pots in a cool greenhouse, in July, October and November. Thus, hatching must have occurred from May onwards, and it was probably not the time of year but some condition of the experiment which prevented hatching in the laboratory.

Hatching experiments thus show that, while potato strain larvae can be stimulated to hatch by excretions from the roots of several species of *Solanum*, the pea, oat and clover strain larvae are not so easily hatched. It may be that at certain seasons of the year they will respond to the root excretions of their host plants.

The results of all the hatching experiments, however, are so variable, even when root excretion and cysts were apparently uniform, and care was taken not to introduce other chemicals into the hatching dishes, that it seems impossible to use it as a means of differentiating strains, unless some method can be evolved whereby reasonably uniform results may be obtained.

Measurements of Larvae.

In view of the variability of the results, the comparatively large amount of material required and the length of time necessary for making infection and stimulation tests, an attempt was made to find out whether measurements of larvae might not give some indication of the strain of a cyst population as a preliminary to the infection tests. Since Schmidt was able to distinguish the oat from the beet strain by larval measurements, it was thought possible that other strains might also show characteristic differences in their larval lengths.

The measurements of larvae were made with the aid of a blood-counting slide graduated in 1/20ths of a mm. The larvae were collected into a small drop of water on an ordinary slide and killed by heat. They were then transferred to the counting slide and, under the binocular microscope, were arranged in turn on the scale and the length determined to the nearest $\frac{1}{4}$ of a division, *i.e.*, to the nearest 1/80th of a mm. When killed by heat the larvae were usually very nearly straight ; any which were bent were not measured. It was found at the start that, if the skin of a

larva was ruptured, part of the contents escaped and the larva immediately became shorter. When this happens, pressure on the larva with the hair-needle, which was used for manipulating the larvae, causes flattening, while uninjured larvae are quite firm and may be rolled about with the hair-needle. Thirty larvae of the potato strain were measured before and after rupture of the skin and were found to shrink on an average by about 10% of their original length. Damaged larvae were therefore not measured.

Owing to the difficulty experienced in obtaining sufficient numbers of hatched larvae of some strains of *H. schachtii* it was decided to measure larvae obtained by dissecting cysts, instead of naturally hatched ones. The cysts were opened with needles under the microscope in a drop of water, and the egg membranes were ruptured by gentle pressure, causing the larvae within to extend rapidly and shoot out of the shells. Larvae which did not straighten on being released were regarded as non-viable and were not measured. The cysts were always soaked in water for at least four days before being dissected. Six cysts were dissected for each strain of eelworm, and 50 larvae measured from each cyst, making a total of 300 larvae for each strain. Ten strains were investigated : four were potato strains, from three different localities in Bedfordshire, Yorkshire and Ayrshire, and one from tomato roots. The other six were strains from peas, oats, sugar beet, clover, *Myosotis* and *H. punctata* from *Agrostis*. All but the three potato strains and the *H. punctata* were removed from the host roots ; the others came from soil in which potatoes and *Agrostis* respectively were infected. For three strains, the Bedfordshire potato, pea and oat strains, 300 hatched larvae were also measured for comparison with the dissected ones.

Table 1 shows the mean larval lengths for the different strains.

It will be seen that there is a considerable difference between the mean length of the smallest and largest larvae. Another striking feature is the difference in length of potato strain larvae from Bedfordshire and from Ayrshire ; this difference is greater than that between the Bedfordshire potato and the pea strains, and between the Bedfordshire potato and the beet strains. The difference between pea larvae obtained by hatching and by dissection is also surprisingly great.

In order to find out whether these differences are significant the figures have been analysed statistically. For the purpose of comparing the various

TABLE 1.

Strain	Mean length of 300 larvae μ	Standard deviation μ	Standard error of mean μ	Variation coefficient %
Oat (hatched)	583·2	28·9	1·6688	4·955
H. punctata (dissected)	581·5	18·338	2·5938	3·154
Oat (dissected)	575·63	22·075	3·1225	3·834
Clover (dissected)	529·41	14·913	2·1075	2·817
Pea (hatched)	483·25	22·925	1·3225	4·734
Myosotis (dissected)	478·91	21·975	3·1063	4·588
Potato [Ayr] (dissected) ...	464·2	15·475	2·1888	3·333
Tomato (dissected)	463·04	23·938	3·385	5·169
Beet (dissected)	462·63	20·913	2·9575	4·521
Potato [Yorks.] (dissected) ...	460·16	12·963	1·8338	2·817
Pea (dissected)	458·88	17·425	2·4638	3·797
Potato [Beds.] (hatched) ...	452·83	27·3	1·5763	6·029
Potato [Beds.] (dissected) ...	452·13	18·963	2·68	4·195

TABLE 2.

Mean lengths of 300 larvae from 10 strains of *Heterodera*.

Strain	n	$\Sigma(x)$	C.F.	Σ(c.f.)	$\Sigma(x^2)$	Σ(s.s.)	Σ(c.f.)–C.F.
Potato—							
[Beds.]...	300	10851	392480·670	393203·3	393879	675·7	722·63
[Ayr] ...	300	11141	413739·603	414226·3	414677	450·7	486·697
[Yorks.]	300	11044	406566·453	407049·16	407372	322·84	482·707
Tomato ...	300	11113	411662·563	412235·02	413313	1077·98	572·457
Pea ...	300	11013	404287·230	405048·06	405619	570·94	760·83
Oat ...	300	13815	636180·750	636697·84	637615	917·16	517·09
Beet ...	300	11103	410922·030	411266·22	412089	822·78	344·19
Clover ...	300	12706	538141·453	538419·8	538838	418·2	278·347
Myosotis ...	300	11494	440373·453	440588·16	441496	907·84	214·707
H. punctata	300	13956	649233·12	649933·16	650566	632·84	700·04
	3000	118236	4703587·325	4708667·02	4715464	6796·98	5079·695

Measurements in 80ths of a mm.

n = number of larvae measured ; 50 each from 6 cysts.

$\Sigma(x)$ = sum of lengths of n larvae.

C.F. = correction factor for $\Sigma(x) = [\Sigma(x)]^2 \div n$.

Σ(c.f.) = sum of the correction factors for each of the 6 cysts.

$\Sigma(x^2)$ = sum of the squared lengths of n larvae.

Σ(s.s.) = sum of squares of deviations from the mean for the 6 cysts = variance within cysts.

Σ(c.f.)–C.F. = variance between cysts.

Mean = 39·412 (80ths mm.) = 0·49265 mm.

Grand correction factor = 4659917·232.

(*Continuation of Table* 2).

Analysis of Variance.

Source	s.s.	Degrees of Freedom	Mean Variance	Variance ratios
Between strains	43670·093	9	V_1 4852·233	V_1/V_2= 47·76
Within strains—				
Between cysts	5079·695	50	V_2 101·594	V_1/V_3=2099·0
Within cysts (error) ...	6796·980	2940	V_3 2·312	V_2/V_3= 43·93
Total	55546·768	2999		

Standard deviation $=\sqrt{2\cdot312}=1\cdot521=19\cdot0125\mu$.

Variation coefficient V=3·859%.

Standard error of mean S.E.$=\sqrt{\frac{2\cdot312}{300}}=0\cdot08778=1\cdot09725\mu$.

Standard error of difference between 2 means $=\sqrt{2}\times1\cdot09725\mu$.
$=1\cdot5525\mu$.

From tables of t when $n=300$ and $P=0\cdot05$, $t=1\cdot96$.

Critical difference between means $=1\cdot96\times1\cdot5525\mu$.
$=3\cdot0429\mu$.

Series	Mean larval length	Differences
H. punctata	581·5μ	—
Oat	575·63μ	5·87μ
Clover	529·41μ	46·22μ
Myosotis	478·91μ	50·5μ
Potato (Ayr)	464·2μ	14·71μ
Tomato	463·04μ	1·16μ
Beet	462·63μ	0·41μ
Potato (Yorks.)	460·16μ	2·47μ
Pea	458·88μ	1·28μ
Potato (Beds.)	452·13μ	6·75μ

strains only the measurements for the dissected larvae are included. The results are shown in Table 2. By analysing the variance the critical difference between the mean larval lengths is obtained. Differences between the means greater than this are significant ; that is to say, they would occur by chance less often than five times in 100 samples of the size taken.

A comparison of the means shows several interesting points. (i) Measurements of larvae of the three potato strains show that larvae

from the same host species grown in different localities may differ significantly in length. (ii) From the mean obtained for the tomato larvae it appears that a change of host does not necessarily result in a very pronounced alteration of the larval length. In order to find out if there is a significant change in larval length with change of host, larvae would have to be measured from cysts developed on the different hosts growing in the same soil. (iii) Larvae of strains which are quite distinct biologically, such as the beet, pea and oat strains, do not necessarily differ significantly in length. (iv) Larvae of *H. punctata* and the oat strain of *H. schachtii* are very considerably longer than those of the other strains measured. The difference can in fact be appreciated, without actually measuring them, when they are observed under a fairly low power of the microscope. Clover and *Myosotis* strains are intermediate between the oat strain and the group of strains with smaller larvae comprising the potato, pea and beet strains. Fig. 1 emphasises this last point.

Another interesting fact which is brought out in the analysis of variance is the remarkably high variation between the mean lengths of larvae from different cysts of the same batch. The ratio of the variance between the cysts to error variance is 43·93, which is highly significant. Since there is so much variation between the cysts, a better idea of the mean larval length of the whole population would probably have been obtained if larvae from a greater number of cysts had been measured. Ten larvae from each of 30 cysts might have been a better choice than 50 from each of 6 cysts. This would not entail a great deal more work, since it is the dissection of the larvae from the eggs rather than the opening of the cysts which takes care and time. In the case of small cysts, such as those of *H. punctata*, it would be easier to get 10 than 50 undamaged larvae from each cyst, as the number of eggs in a cyst is often little more than 50.

The comparison of the measurements of hatched with dissected larvae is given in Table 3.

In this analysis no allowance is made for variance between cysts, since this, while complicating the analysis, was found to make no appreciable difference to the final results. It will be seen that the differences between hatched and dissected larvae of both oat and pea strains are highly significant, but not so in the case of the Bedfordshire potato strain. It is possible that a certain amount of swelling or stretching of the larva occurs after it has hatched, perhaps through the absorption of water;

or, possibly, small larvae which are incapable of hatching, or slow to do so, occur in the cysts and are released by dissection only. Graphs of lengths

TABLE 3.

Comparison of Hatched with Dissected Larvae.

Strain	n	$\Sigma(x)$	c.f.	$\Sigma(x^2)$	Σ(s.s.)
Potato (Beds.) Hatched...	300	10868	393711·413	395138	1426·587
Pea ,, ...	300	11598	448378·680	449384	1005·320
Oat ,, ...	300	13997	653053·363	654651	1597·637
Potato (Beds.) Dissected	300	10851	392480·670	393879	1398·33
Pea ,,	300	11013	404287·23	405619	1331·77
Oat ,,	300	13815	636180·75	637615	1434·25
	1800	72142	2928092·106	2936286	8193·894

Mean larval length $40·0789 = 500·9863\mu$.

Correction factor 2891371·202.

Analysis of Variance.

Source	s.s.	D.F.	Mean Variance	Ratio of Variances
Between strains	36720·904	5	7344·181	1608
Within strains (error)	8193·894	1794	4·566	
Total	44914·798	1799		

Standard deviation $= \sqrt{4·566} = 2·136 = 26·7\mu$.

Standard error of mean $= \sqrt{\frac{4·566}{300}} = 0·1234 = 1·5425\mu$.

S.E. of difference between means $= \sqrt{2} \times 1·5425 = 2·18125\mu$.

Critical difference $= 1·96 \times 2·18125 = 4·27625\mu$.

Strain	Mean larval length	Difference
Oat hatched	583·208μ	
Oat dissected	575·625μ	7·583μ
Pea hatched	483·25μ	
Pea dissected	458·875μ	24·375μ
Beds. potato hatched	452·833μ	
Beds. potato dissected	452·125μ	0·708μ

of larvae obtained by cyst dissection, however, give no definite indication of the presence of a *minor* form of larva in the oat or the pea cysts, such

as that described by Schmidt for the oat strain. It is curious that no appreciable difference between hatched and dissected larvae should be shown in the potato strain.

In order to try to account for the variation which was shown to occur between lengths of larvae from different cysts of the same strain, the correlations between larval length, cyst size and number of eggs in the cyst were calculated for 20 cysts from the four potato strains. None of the lemon-shaped strains was included on account of the difficulty of calculating their volume. An estimate of the size of the rounded cysts was arrived at from measurements of the length and breadth of each cyst made before it was dissected. One-quarter of the sum of the length (excluding the neck) plus the greatest breadth was taken as representing the radius of the approximately spherical cyst.

As would be expected, a high positive correlation was shown between cyst size and number of eggs per cyst. The value obtained for the partial correlation of these two characters would occur by chance in considerably less than 1% of similar samples of 20 cysts. The total correlation of cyst size and larval length showed a value which would occur by chance in 2% of similar samples, but when the partial correlation of these two characters was worked out, eliminating the correlation due to the number of eggs, the probability of its occurring by chance was increased to 10 %. It is therefore not considered significant. No significant correlation was found between the number of eggs and larval length in a cyst. These results are set out in Table 4.

Table 4.

Correlation	Correlation coefficient	Probability
Total correlation :		
Cyst size and larval length	·5163	·02
Number of eggs and larval length	·3791	·1
Number of eggs and cyst size	·6871	·001
Partial correlation :		
Cyst size and larval length ... ... (No. of eggs constant)	·3801	·1
Number of eggs and larval length ... ... (Cyst size constant)	·03917	above ·1
Number of eggs and cyst size ... ... (Larval length constant)	·6201	between ·01 and ·001

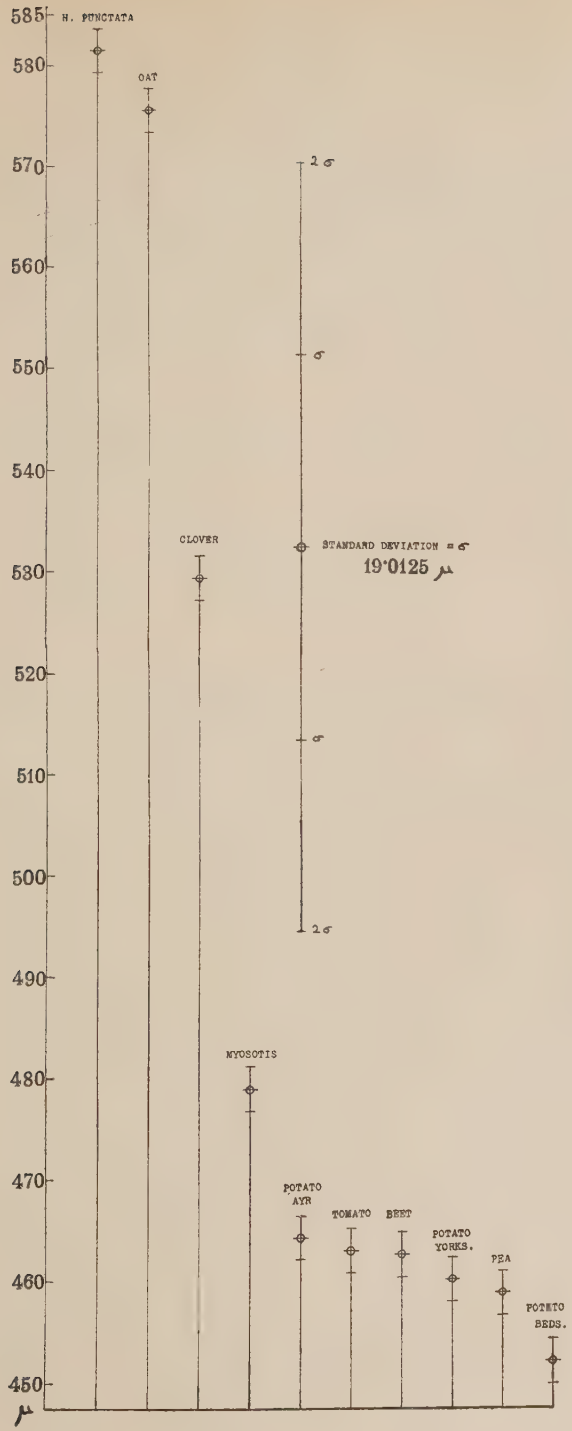

Fig. 1. Differences in mean larval length of 300 larvae of 10 strains of *Heterodera*. Mean marked thus ⊖. A distance equal to twice the standard error of the mean (=2·1945μ) is marked off above and below this point.

Discussion.

Of the different means of identification of *Heterodera* cysts which have been explored, that of testing the powers of infection of the cysts when exposed to growing plants remains for the present the only one possible for most of the lemon-shaped strains. It is a method which must be used with care, since it is easy accidentally to introduce cysts of different strains into the experiments. In addition, results must be confirmed several times before they can be regarded as established.

With regard to the hatching of larvae from cysts as a means of identifying strains, it appears unlikely that a satisfactory method of differential stimulation can be evolved for all strains, since many of them refuse to hatch during at least part of the year, and the degree of hatching from apparently similar cysts has proved to be exceedingly variable. Possibly when more is known of the response of the larvae of the various strains at all seasons and to different stimulants, it will be possible to differentiate strains in this way.

By means of measurements of larvae it may prove possible to identify *Heterodera* populations without the need for infection experiments, or at least to get an idea as to the identity of the strains, in order to reduce the number of host plants to be tested. On comparing the mean lengths of 300 larvae for the ten strains measured it appears probable that, given the mean length of 300 larvae of an unknown strain with lemon-shaped cysts, one could be fairly certain as to whether it was oat strain, clover strain, or belonging to the group with smaller larvae, i.e., beet, pea and *Myosotis* strains. Before one can be quite certain of this, however, it will be necessary to find out the variations which may be expected between the mean lengths of larvae of each strain from different localities. The differences between the means for the three potato strains are not very great as compared with the difference between the potato and oat strains, but three strains is not a sufficiently large number on which to base the conclusion that these differences are never very great. Schmidt gives the mean larval length of a Swedish potato strain as 497 μ, which is considerably higher than that of any of the potato strain larvae recorded here. It is, however, possible that some *H. punctata* cysts were included with his potato strain cysts, as it was not then known that this species is frequently found in the soil. It is possible that the variation between oat strains from different parts of the country may be much

greater, or much less, than that between potato strains. However, the value given by Schmidt for the mean larval length of oat strain larvae in Germany is 0·575 mm. as compared with 0·5756 mm. now obtained for the English oat larvae. His figure for the beet strain is not quite so close to that found for the English beet strain measured here : it is 0·47 mm. as compared with 0·463 mm. Many more measurements are necessary before a correct idea can be gained of the variations likely to occur between different populations of the same strain.

Another factor which requires careful investigation is the mean larval length of the same strain from different hosts. If there is considerable change with change of host, the possibility of identifying strains by means of larval measurements may be much reduced. In the one example given here of larval lengths of the same strain from different hosts there is no great alteration in length, but, as pointed out before, for valid comparison the different hosts should be infected from the same source.

The variation in the mean length of larvae from different cysts is of some interest. Since no correlation could be shown between larval length and cyst size, one wonders whether the larval size is in any way related to the host plant. It may be that, on host plants which are sickly the female nematodes develop less strongly, and produce smaller larvae than those produced by nematodes on healthy hosts. If larval size is really influenced by the health of the host this may account for the variation between larvae from different parts of the country, since the host plants are almost bound to vary in vigour to a certain extent in different places. The variation in different localities may also be influenced by the soil and climatic conditions, the processes in the cultivation of the infected crop, or the variety of the host plant.

If, on further investigation, the larvae of the oat strain of *H. schachtii* prove to be invariably so much longer than those of all the other strains, as would seem to be the case, the validity of Schmidt's *major* subspecies is upheld. The *minor* subspecies is, however, not distinct, as a fairly wide range is covered by larval lengths of the pea, beet and *Myosotis* strains. The clover strain is intermediate between oat and *Myosotis*, and it is possible that when a greater number of strains has been investigated more intermediate forms will be found, so that no hard-and-fast line can be drawn between *major* and *minor* larvae.

It is now quite clear that *H. punctata* differs sufficiently from the lemon-shaped strains of *H. schachtii* for there to be no doubt as to its validity as a species. The main points of difference are : the rounded cysts with punctation-like markings in rows on the walls, the anal aperture approximately equal in size to the vulva, large larvae, approached in size only by those of the oat strain, with longer and more slender tails. In addition to these morphological distinctions are two biological characters. These are the pronounced susceptibility of the cysts to drying, not met with in *H. schachtii* to anything like the same extent, and a peculiarity observed during the dissection of cysts, namely the presence in many of them of eggs at all stages of development. In *H. schachtii* the eggs in a cyst have always been found to be at practically the same stage. In his description of *H. punctata* Thorne states that the eggs in the cysts he examined were equally developed ; this may have been due to chance, or to the season of the year when they were examined. The cysts containing eggs at all stages of development were removed from the soil in March.

The potato strain of *H. schachtii* is in some ways intermediate between the other strains and *H. punctata*. It resembles the latter in having no prominent vulva and in having rows of punctate-like markings on the cyst wall. It differs, however, in having no well-marked sub-crystalline layer, in having smaller larvae without very slender tails, in the very small anus, in the much greater resistance of the cyst wall to drying, and in having all the eggs at the same stage of development in a cyst. It differs from the lemon-shaped strains in the shape of the cyst, in having rows of punctation-like markings on the cyst walls, and from most of them, possibly from all, in the absence of a conspicuous sub-crystalline layer. It also differs from some of the lemon-shaped strains in its marked reaction to the root secretions of certain plants.

Although claims have been made by several workers, notably by Zimmermann (1927) and Goffart (1928), that the potato strain can be made to infect sugar beet, and in doing so that the cysts take on the lemon form, these results are not generally accepted, since other workers have been unable to repeat them, and the beet strain has never been made to infect potatoes. It is therefore very unlikely that the potato and beet strains are identical. On the contrary, the differences listed above between the potato strain and all the other strains provide strong grounds in support

of Kemner's designation of the potato strain as *H. schachtii* subsp. *rostochiensis*.

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Summary.

1. Three methods of differentiating the various strains of *Heterodera schachtii* are tried out in an attempt to find a quick means of identifying the strains.

2. Infection experiments using possible host plants, though they are the final test of strain, require careful handling and take some weeks to carry out. In a limited number of such experiments a strain of eelworm occurring on wild clovers appears to be distinct from the pea, oat and beet strains. A strain parasitic on *Myosotis* appears to be distinct from the clover strain.

3. Attempts to stimulate the hatching of larvae from cysts by means of root excretions of host plants were unsuccessful in the case of oat and pea strains. Potato strain larvae appear to be stimulated by root excretions of several solanaceous plants.

4. Measurements of larvae show significant differences in mean lengths between some strains, between samples of the potato strain from different districts and between different cysts of the same strain. Of the strains measured the oat strain larvae and those of *H. punctata* stand out as being considerably longer than the others.

5. The potato strain is regarded as differing sufficiently from the others for Kemner's designation of it as *H. schachtii* subsp. *rostochiensis* to be upheld.

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The Effect on Seed Potatoes of Formalin Treatment for the Destruction of Adherent Eelworm Cysts.

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Laboratory experiments have shown that cysts of the eelworm *Heterodera schachtii* adhering to seed potato tubers are killed by immersion of the tubers for 6 hours in 5% formalin solution (Franklin 1939). Further experiments were designed to determine what effect such treatment has on the seed tubers and on the resulting crop.

(1) *February Experiments.*

On February 10th, 1939, 260 tubers (about 50 lbs.) of the variety *Majestic* were soaked for 6 hours in a 5% solution of commercial formaldehyde. The tubers, on removal from the solution, were rinsed in clean water and set out in sprouting boxes, where they dried satisfactorily. An equal number of untreated tubers was set out for sprouting at the same time. On April 8th two of the untreated tubers and 7 of the treated ones had failed to sprout. The treated tubers were rather softer than the untreated, and somewhat pitted: most of those which had been damaged before treatment had failed to grow, probably on account of the entry of formalin through the wounds. At the time of planting on April 24th, 247 treated tubers were considered fit to set. All but one of these were planted in three rows of 82 tubers each, and an equal number of untreated tubers was planted in three rows arranged at random with the rows of treated tubers. The whole plot was surrounded by control tubers.

During growth no appreciable difference was observed between the rows.

By August 25th the haulms had all died down on account of potato blight, and the crop was lifted on August 28th. The total yield from each row was weighed, riddled over a $1\frac{3}{4}$ inch sieve, and the ware weighed separately.

The yields were as follows :—

	Untreated lbs.	Treated lbs.	Difference lbs.
Mean total yield per row	129·08	132·08	+3·0
Standard error	2·70	11·36	11·67
Mean yield of ware per row	90·42	85·17	−5·25
Standard error	1·96	9·31	9·51
Mean yield of chats per row	38·67	46·92	+8·25
Standard error	0·87	2·68	2·82
Mean percentage ware per row ...	70·03%	64·24%	−5·79%
Standard error	0·29	1·75	1·78

The results showed no significant difference in the total yield or the yield of ware alone between treated and untreated tubers. The yield of chats was, however, significantly greater from the treated tubers, and the percentage of ware was significantly less than in the untreated. It must also be noted that, while 13 treated tubers failed to grow, there were only two failures among the untreated.

(2) *December Experiments.*

Tubers of three varieties were treated on 7th December, 1939, with 5% formalin solution for 6 hours in the same way as in the previous experiment. The varieties were *Arran Pilot* (early), *Ally* (second early) and *Arran Banner* (maincrop). Three hundred sound tubers of each variety were chosen, amounting to about half a hundredweight. After steeping in the formalin solution at a temperature of 7–8°C., the tubers were rinsed in cold running water, stacked in heaps to drain overnight, and then set out in boxes to sprout. An equal number of untreated tubers was set out at the same time.

At the beginning of January, although sprouting had not started in any of the varieties, it was obvious that the *Arran Pilots* had suffered some damage as a result of the treatment. The tubers were softer than the controls and somewhat pitted. Ten tubers had rotted. The variety *Ally* was less severely affected ; there was some pitting of the tubers, and 7 were partly rotten. The late variety, *Arran Banner*, on the other hand, was practically unaffected.

During the next two months sprouting took place. The earlies and second earlies were a little retarded by the treatment, but *Arran Banner* still appeared to be unaffected. On 18th March the tubers were examined

carefully. The treated tubers of *Arran Pilot* had sprouted almost as well as the controls, but they were rather badly pitted and definitely more shrivelled and softer than the untreated. Ten were dead, making a total of 20 tubers probably destroyed by the treatment, or 6–7% of the total number treated. Two control tubers had failed to grow.

There was also little difference between the size of the sprouts in control and treated *Ally* tubers, but the treated tubers here again were softer and more shrivelled than the untreated. Twenty were dead, which, with the 7 recorded before, makes a total of 9% probably killed by the treatment. Two control tubers had failed to grow, but they were not soft and shrivelled as were the treated ones.

Arran Banner alone showed no ill effects after treatment. None of the treated tubers was dead, though one of the controls had failed to grow. The rest showed normal healthy sprouting without obvious deterioration of the tubers.

It remains to be seen whether the pitting of the tubers will have any adverse effect on the growth and yield of the crop. From last year's experiment it appears probable that tubers which have sprouted fairly normally, even though somewhat shrivelled, may produce a normal crop.

It is obvious from these observations that great care must be exercised in treating seed potatoes with 5% formalin, and much experimenting must be done before the treatment can be used generally. Although *Arran Banner*, treated in December, seems to have suffered no damage, and *Majestic*, treated in February, was little harmed, *Arran Pilot* and *Ally* were somewhat damaged, though treated early in December. For early and second early varieties it is possible that treatment in October might be harmless.

As the object of the formalin treatment of seed potatoes is to kill potato eelworm cysts which may be carried in the soil adhering to the tubers, the potatoes used in the experiments were examined to discover whether any cysts had been brought in with them. The tubers were lightly brushed and the soil obtained, together with soil shaken from the sack containing the seed, was sieved to remove the larger pieces of straw, sacking and other débris. The sieved soil was then shaken up with water and the floating material examined for cysts. In this way 450 gms. of soil were obtained from the *Majestic* tubers used in the first experiment. In it were found 73 lemon-shaped cysts and 1 rounded one. The latter was examined to see whether it was of the potato strain or the strain

found on *Agrostis* (Franklin 1938), but it proved to be empty and too much damaged for identification. From the *Arran Pilot* tubers there were 97 gms. of soil containing 20 lemon-shaped cysts and 2 rounded ones of the type found on *Agrostis*. From *Ally* 82 gms. of soil contained 9 lemon-shaped cysts, and from *Arran Banner* there were 258 gms. of soil with 87 lemon-shaped cysts and 21 rounded ones. Of the latter 16 were of the *Agrostis* type, 3 were too much damaged for any accurate idea of their origin to be formed, and 2 might have been of the potato strain. These two were empty, and under a low powered microscope it was not possible to see clearly the anal and vulval apertures as is usual in the *Agrostis* type of cyst. The cyst wall was, however, rather thinner and paler than normal for the potato strain, though this may have been due to the age of the cysts. Thus, since the lemon-shaped cysts are not parasites of the potato, no cysts were discovered which would harm a potato crop, but it is again demonstrated that cysts may easily be carried on seed potatoes, and therefore that seed produced on land infected with the potato eelworm will spread infection wherever it is planted, unless it is first treated for the destruction or removal of adhering cysts.

The cost of steeping seed potatoes or eelworm infected bags, sprouting boxes or other material in 5% formalin is not great. In the experiment described here 800 cc. of commercial formaldehyde were used for $\frac{1}{2}$ cwt. of seed. The cost of the chemical would be 28–30 shillings per ton of seed. It is possible that the solution could be used repeatedly, which would greatly reduce the cost, but this point has not yet been determined. The only other equipment necessary for the treatment is a large bath and a hose to wash down the tubers after treatment. The tubers could probably be steeped in sacks, or preferably, in the sprouting boxes.

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On the Helminths of Corvid Birds in the British Isles.

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In 1925 Chapin reviewed the genera *Syngamus* and *Cyathostoma* and in the review described a new species of *Syngamus* which he had obtained from the American Crow, *Corvus brachyrhynchos* : this, he called *Syngamus gracilis*. It had been found in a bird from the Zoological Gardens at Philadelphia. He was of the opinion that this species had probably been recovered before from the American Crow but that its specificity had not been recognised. He assumes, in fact, that some if not all of the records of the occurrence of *S. trachea* in this species of host, and by inference probably in other Corvidae also, are incorrect and that *S. gracilis* is the species found. He bases this assumption on the physiological reactions of the parasite. It is a well known fact that *S. trachea* does not establish itself well in chickens and can in fact only reach maturity in young or very sick birds. The turkey is generally assumed to be the natural host of this helminth and Chapin is of the opinion that if *S. trachea* cannot establish itself satisfactorily in the chicken, then it is highly improbable that it can do so in the crow. For the chicken and the turkey are closely related phylogenetically, both being gallinaceous birds while the crow belongs to the Corvidae, which has no near kinship with the Galliformes.

Specimens of a species of *Syngamus* have frequently been recovered from Corvid birds in England and I have had the opportunity of examining parasites from a variety of hosts belonging to the Corvidae—mainly from the British Crow, from magpies and in great numbers from the rook and it has been established beyond doubt that the Corvidae of the British Isles are parasitized by *S. trachea* to a very great extent. Not only do individual birds carry the parasite in large numbers, but a very high percentage of the birds examined were infected to a greater or less degree, though the degree of infection varies at different times of the

year. Further facts of the seasonal distribution of the helminth will be given later. Moreover infection is not confined to a single host species, for at least five species are involved from the writer's personal experience. They are the rook, jay, jackdaw, crow and magpie.

S. trachea and *S. gracilis* are not difficult to distinguish though unfortunately original material of the last named species was not available for actual comparison. However, the figures and the description given by Chapin are clear and easy to follow. The males show the most marked and definite differences and with a little patience and care it is not difficult to isolate the male from the female by the use of a pair of sharp needles and dissecting microscope. Identification can then be made on the structure of the bursa and spicules. In the past some stress has been laid on the size of the worm, the flexing of the head and on the positions of the vulva and anus but these vary relatively at different ages and cannot therefore be relied upon for identification. Chapin describes the bursa and spicules as follows :—narrow and deep ; branches of the dorsal ray 150μ long ; externo-dorsal ray slightly shorter, measuring 132μ. The lateral rays are mutually contiguous, parallel and about the size of either branch of the dorsal. The ventral rays are smaller and slender. The spicules are distinctly unequal ; the right is bent and measures 79μ long while the left is nearly straight and is but 69μ long. His drawing shows that the dorsal ray is deeply bifid and each branch is entire and undivided. In the case of *S. trachea* the dorsal ray is deeply bifid also but each branch is typically trifid. Some variation tends to occur in different individuals in these secondary branches and the result may even be markedly asymmetric. The spicules measure from 57μ to 64μ in length. Another species of *Syngamus*—*S. microspiculum*—has been recorded by Skrjabin in Russian Turkestan from *Phalacrocorax carbo*, also a corvid bird. This can, however, easily be recognised by the small size of the spicules. The measurements given by Skrjabin himself vary in different papers but there seems no doubt that they are very short and measure about 49μ long. This is a very much smaller size than occurs in any other species of the genus. The dorsal ray of the bursa is bifurcate at the tip only and each branch is simple.

Thus it will be seen that identification of the species of the genus *Syngamus* from Corvid birds presents no unusual difficulties.

Syngamus trachea has a wide range of possible hosts. It has been recovered by the present writer from the rook *Corvus frugilegus*, the

carrion crow *C. corone*, the jackdaw *C. monedula*, the magpie *Pica pica*, the jay *Garrulus glandarius*—all corvid birds—from the common starling *Sturnus vulgaris* and from a number of gallinaceous birds, in particular the red grouse *Lagopus scoticus*, the capercaillie *Tetrao urogallus*, the red legged partridge *Alectoris rufa*, the chukar *A. graeca chukar*, the common partridge *Perdix perdix*, the pheasant *Phasianus colchicus*, the domestic fowl *Gallus gallus* and the turkey *Meleagris gallopavo*.

The turkey is probably the natural host of this parasite, but of the others cited above, only the domestic fowl shows any marked degree of resistance. All the other hosts have been found infected at a variety of ages, usually, however, most frequently so when young.

Infection in many cases occurs very early in life. Starlings and rooks are certainly infected in the nest. Recently some young rooks were removed from the nest before they were fully able to fly and they were found to contain from 2–6 pairs of gapeworms in the trachea and these were mature and contained fully viable eggs. A similar condition has been found in nestling starlings by Morgan (1931). Juvenile rooks which had begun to leave the nest but which still returned to it, frequently contained much heavier infections, one having as many as 53 pairs of worms. Older birds were by no means clean though not every bird was infected as is the case among the nestlings and juveniles. About 54 per cent. of the older birds shot during the winter months from January to March contained infections varying from one pair to 12. It is probable that the young are very susceptible to infection. Yet another factor that has to be taken into consideration, however, in this respect is that the nestlings are fed almost entirely on small earthworms and on insects and their larvae—caterpillars, beetles and the like—while the older birds, particularly in the later parts of the year take, not only animal food but large quantities of vegetable food also, mainly grain. It may be therefore that the number of larvae picked up varies inversely with the age of the bird and the seed concentration.

Though several hundreds of bursae have been examined, *S. trachea* only has been found and it seems certain therefore that British corvid birds normally carry this parasite. It would be interesting to have more details of the parasites of corvid birds in America. That gapeworms from rooks and similar birds are readily transmissible to the gallinaceous birds has been shown many times in this laboratory and in view of the fact that no foci of infection of gapeworm from Galliform birds are now

available to the writer, rooks are indeed used as the main source of material for experimentation.

It has been noticed that the gapeworms in the trachea always lie with the anterior end facing down towards the bronchi and with the tails pointing upwards towards the mouth. This is the case not only with those found in rooks but also in every bird examined. Only rarely does one find a worm attached in the other direction. This position is probably taken up because the force of expiration is greater than the force of inspiration and probably has no bearing on the physiological requirements of the parasite itself. It is customary to find the male worm strongly attached to the trachea, while the female is free.

The degree of infection with other helminths among nestlings of rooks is interesting for a number of parasites have been recovered from them. Practically all the birds contained *Capillaria ovopunctata* : one had as many as 13 specimens ; a single bird had a young *Porrocaecum ensicaudatum*. Rather more than half carried young forms of *Hymenolepis spp.*, but as none of them was more than 7 mm. long and as the original terminal segment was still present they must have represented recent acquisitions. Occasional specimens of *Harmostomum sp.* have been found. The juveniles also carried the same parasites, usually in greater numbers. *Porrocaecum* was still the rarest parasite but it occurred in about half the birds. *Capillaria* became very abundant and the cestodes too were more frequent and showed greater development. In these birds also there was greater variety of infection. Many of them carried 3 different species of helminths while in the infections of the nestlings, infection was often limited to a single species, rarely 2 and never 3. Two species of *Hymenolepis* have been obtained, one of which was certainly *H. serpentulus*. One bird carried 37 scolices and one length of fully developed segments ; the others were young specimens. None of these birds had any *Acanthocephala* though old ones shot at the same time were infected with them.

One interesting fact is that not all these birds carried identical infections though they were limited to two small rookeries not more than 600 yards apart and the parent birds were all feeding locally in the same small district. Yet the resulting infections showed marked differences in different birds ; for instance, of a pair of birds taken from the same nest, one bird carried *Capillaria* only, while the other had *Capillaria* and many cestodes in two different stages of development. They had not all been taken in at the same time. In another case one bird had an infection of

Porrocaecum and its mate had none. It seems strange that *Porrocaecum* was not met with more frequently for it is very abundant in older birds in this district. The absence of *Acanthocephala* may be due to its need for an intermediate host, which possibly appears later in the year.

Other rooks have been examined from time to time from other districts ranging from Hertfordshire to Oxfordshire. They have all contained the same parasites as those recovered from the local rookeries. This is not surprising as adult rooks normally fly far afield during the day, only collecting together at night. In this way the helminth parasites would be widely spread.

The gut contents of these rooks have been examined not only for adult helminths but also for ova. The débris was washed and the eggs concentrated by centrifuging in zinc sulphate solution, the specific gravity of which was 1·25. Numerous helminth ova were recovered and the samples contained not only ova from the helminths of rooks but also eggs from other helminths. There were for instance eggs of some *Taenia sp.* which it has been impossible to identify more closely. But they were recognised by the presence of a radially striated covering and by the presence of a hexacanth embryo. There were also larger ova closely resembling those of *Heterakis gallinae* or *Ascaridia lineata.* Not every sample of gut contents contained alien eggs and when such eggs did occur, it was usual to find only single specimens which made identification well nigh impossible. But on one occasion when a large quantity of faecal matter had been examined, there were recovered 9 eggs closely resembling *Heterakis gallinae* ova. These were left in water at room temperature and development occurred giving rise to moving embryos. These embryonated eggs were fed to a young chicken which had been reared indoors under parasite-free conditions and at post-mortem examination five weeks later, 5 specimens of *H. gallinae* were obtained. These eggs had no doubt been ingested with soil attached to some small insect or earthworm and had survived the passage through the gut of the rook to complete their development later on their return to normal environments. This observation is interesting in view of its practical bearing for it shows one way of spreading helminths to clean pastures and may explain why domestic birds acquire helminthic infections when they are put on to clean pastures.

Faeces collected from the ground under rookeries have also been examined for helminth ova. Eggs closely corresponding to those of

Heterakis gallinae have been recovered on two occasions but it has been impossible to put these to the final test of feeding for exact identification. In these examinations there is always the danger that the faeces may have been contaminated with ova after they have reached the ground, but after the actual feeding experiment described above, there can be little doubt that rooks may pick up eggs of parasites of domestic birds, discharge them unharmed after passage through the gut and thus spread the parasite and possible disease to fresh pastures.

On many occasions soil which had been found attached to the feet and beaks of birds has been examined for helminth eggs but, so far, it has not been possible to establish definitely that such material is a source of infection.

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Further Studies on *Coenurus glomeratus*.

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Budding and vegetative increase is a well established feature in many larval cestodes. In Echinococcus it may be said to reach the highest peak of efficiency. It has also been recorded by Leuckart in 1886, when he examined a specimen of *Cysticercus tenuicollis* which apparently contained a few isolated daughter bladders within the mother cyst, but unfortunately he does not draw them or describe them in detail so it is impossible to say if the daughter cysticerci were complete with a scolex and what exactly was the number which had developed in the parent cyst. It is unusual to find daughter cyst formation among the cysticerci and so far as I know this is the only record, but it is well known among coenuri. It occurs so frequently in *Coenurus serialis* as to be considered almost normal. Both external and internal daughter bladders have been found. In *Multiceps glomeratus* a single larval infection showed a vast degree of budding. Some of the original cysts were simple being more or less regularly spherical or ovoid, but many of them carried a large number of exogenous daughter outgrowths which were still attached to the maternal cyst by means of a stalk. Some of these outgrowths may have been due to local pressure by the viscera or other organs but a number of them had arisen in positions where there was no obvious pressure and no apparent reason for such development. There were also in some individual adventitious cysts, small coenuri quite free from the mother cyst but one hesitates to say definitely that these were true daughter cysts formed by budding because it is just possible that they were developed independently from a hexacanth embryo and had become enclosed in the same host cyst with another parasite. Internally some of these coenuri carried daughter coenuri, none of which, however, was attached to the original cyst by a stalk, but were completely free, floating in the fluid. These cysts could not have arisen independently from embryos as they were inside another coenurus and must therefore by some means have arisen from the parent coenurus. The mechanism of their origin is not entirely clear. What seems certain, however, is

that they cannot have arisen by simple invagination from the mother cyst wall and subsequent pinching off. Had this been so, the cuticular layer would then have been on the inside of the resulting daughter cyst forming a lining which would have effectively prevented the development of scolices. This budding method has, however, been assumed by Railliet in 1895 and by Neveu-Lemaire in 1936 for the development of endogenous daughter cysts in *Coenurus serialis* but it is not easy to see how the orientation and organisation could become normal without also assuming either a complete invagination and this is not easy to visualise or a complete destruction of the cuticle followed by re-growth elsewhere. This would be a very unusual procedure for cuticle is very persistent and cells do not readily change their functions. There has been found no evidence for simple invagination methods of internal daughter cyst formation, during the examination of many sections from these cysts. It is therefore assumed by the present writer that some method other than simple budding has been adopted and that therefore exogenous and endogenous daughter cysts are not strictly homologous.

However, it is certain that exogenous budding does occur in *C. glomeratus*. It has often been seen and sketches are given of a portion of one coenurus showing to what a marked degree it may be present (Figs. 1 and 2). It may be remarked now that not all of these pedunculated cysts were gravid and contained scolices. The smaller ones were actually solid, the internal cavity being filled with typical platyhelminth parenchyma. This was much finer and more vacuolated than is usually found but will be described later in this article.

It may first be well to consider the histological structure of the typical coenurus of *M. glomeratus*. On the outside is a layer of cuticle varying somewhat in thickness. It is not smooth but is thrown into folds and papillae giving a rough appearance to the surface of the coenurus when examined under magnification. Beneath this is found a nucleated layer which secretes the cuticle towards the outside. This layer is probably cellular, the cells being of an elongated fusiform shape but cell outlines are sometimes difficult to distinguish. Beneath this there seems to be a very fine layer of muscle fibres running mainly tangentially but also passing centripetally and centrifugally into the adjacent layers. Under this is a well marked layer of parenchyma about 150μ thick but not being entirely regular. In places it is as thin as 100μ ; in others it reached 175μ. It consists of nucleated cells drawn out into fibres enclosing large

numbers of intercellular spaces. There is no definite cellular layer delimiting the wall from the central cavity. This is the state of affairs found in the typical adult mature coenurus with developed scolices, but something rather different can be seen in young ones and in the small evaginations from the old ones. Here there is no central cavity ; instead it is occupied by a very thin and delicate connective tissue parenchyma.

Fig. 1.—A complete coenurus of *Multiceps glomeratus* showing budding.
Fig. 2.—A portion of exogenous budding showing the fine ramifications and some developing scolices.

The intercellular spaces are large and the cells and their fibrous processes are long drawn out forming a very loose vacuolated network, the whole being filled with a fluid, some of the physical characters of which have already been described in a previous paper.

The beginning of the scolices can be picked out very early in the growth of the coenurus. To the naked eye they appear as slightly opaque patches on the surface of the wall. In section they are shown to have the following structure. A region develops in the outer layer of parenchyma where the intercellular spaces tend to become occluded. The nuclei

and the cells become more prominent and take up the stain more deeply than the surrounding tissues. The whole layer becomes wider at the expense of the very thin internal parenchyma. At the same time a certain amount of invagination occurs from the surface, which deepens steadily and continually. Meanwhile differentiation of the head tissues is occurring until finally suckers, hooks and rostellum can be picked out. The invagination from the surface is complete and the whole scolex structure can be recognised. All this time, however, the loose internal parenchyma has been regularly encroached upon but it still remains intact and the young bladder is still solid. As, however, development of the scolex proceeds and growth of the coenurus continues, extreme pressure is brought to bear upon the fine tissues and rupture occurs and in this way the central cavity starts. The other layer of parenchyma just under the cuticle-producing cells is tougher and withstands considerable force.

Some of the scolices seem to develop in close proximity to each other in blocks of three or four, and right up to the stage when typical hooks can be seen, the main bodies of the scolices are not independent of each other but are still united by means of the parenchyma (Fig. 3). Fibres can be seen under oil immersion magnification which pass distinctly through the tissue and fuse the developing heads together into one mass. Presently, however, further growth pressure comes to bear upon the parenchyma and this finally ruptures between the scolices. Not until this occurs are the heads found floating freely in the fluid and not until then can they be considered as mature. Scolices in various stages of development have been fed to dogs from time to time. Not all infections gave positive results and it is possible that some of the experimental feedings were made with these immature heads for the state of maturity was at first assessed by the hook development as seen under a fairly low magnification.

It is suggested that only a small portion of the adult coenurus retains its reproductive function and is capable of producing new scolices and that it is this region that has the power of budding to form new young daughter coenuri which presumably are more vigorous than the ageing parent bladder. Budding would seem to be a means of overcoming increasing age and sterility in the coenurus. Old age seems to set in, in this species of coenurus, about 16–18 months after the original feeding has taken place. This time factor is taken from observations when the rabbit is used as host ; it may be different when other hosts are observed.

In some coenuri were seen scolices of a dusky pink colour. These tended to be vacuolated and stalked and some had even broken away from the wall of the parent cyst and were floating quite independently in the fluid in the central cavity. It was thought at one time that they were perhaps stages in the development of endogenous daughter bladders but after examination of many sections of such pink scolices, both free and still

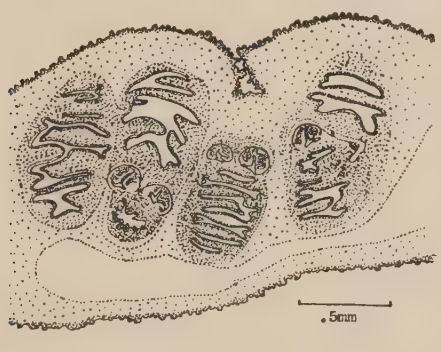

Fig. 3.—Cross section of young coenurus showing the development of 4 scolices in a block. Tearing of the parenchyma has just begun as the result of growth pressure.

attached, an opposite conclusion has been drawn. Much vacuolation had occurred in the parenchyma and there was evidence that the suckers were undergoing degeneration. Furthermore, in the invagination canal there was an accumulation of débris and mammalian leucocytes. Polymorphonuclears and macrocytes were especially abundant, which facts probably indicate some phagocytic action. Eosinophiles were present in small numbers.

Implantation.

A number of scolices and young coenuri were transferred to other rabbits. Some just degenerated and left no trace but at least one

continued its development. One small cyst measuring about 1 cm. in diameter was placed under the skin under the right scapula. During the operation it was punctured and collapsed. No further development occurred. A portion of cyst wall bearing a group of about 20 mature scolices was placed in a similar position in another rabbit. This too did not proceed any further with its development. The same negative findings resulted when groups of scolices were transplanted under the skin in the left groin of rabbits. But a portion of a coenurus bearing a group of very young scolices continued its development when transplanted under the skin of the neck of another rabbit. For 3 months there was no obvious signs of life but then a small lump appeared which gradually grew until it reached a diameter of about 3 cm. As it then seemed as if it were getting smaller, a post-mortem examination was made and a typical coenurus of *M. glomeratus* was found. It contained 47 scolices which were fertile to dogs.

This single positive result shows that regeneration and regrowth can occur in the coenurus of *M. glomeratus*. Efforts are being made to work out the changes which occur but difficulties arise because not all rabbits make suitable hosts for the parasite.

This result can be compared somewhat with what happens in echinococcus infections. Rupture of a hydatid, when some of the elements leak out, often leads to an increased infection. Some of the contents of the cyst, if not too far advanced in their specialisation can form new cysts in whatever site they find themselves, Just which elements have this power is not clear, but evidence is piling up, chiefly from Dévé and Dew, that both the germinative membrane and the young scolex can adapt themselves to new conditions and give rise to new growths.

An interesting observation was made following the infection of a dog with *Multiceps glomeratus*. Over a period of several weeks the dog had shown that it was free from all cestodes as judged by faecal examinations. It was fed one complete and intact coenurus. This, when held up to the light was translucent and it could be seen that there were no daughter bladders enclosed. When the dog was slaughtered 3 months later, 114 specimens of adult *M. glomeratus* were recovered from the small intestine. Of these 101 were typical ; 12 had 6 suckers and a single rostellum and the other had 8 suckers and one rostellum. However, while the typical specimens were long and fully mature, the monstrosities were stunted. The greatest length attained by any of them was only

7 mm. Segmentation was only just beginning and it could just be seen that all the strobilae would have been tri-radiate. Though the whole bulk of parasitic material was searched closely, no developed segments could be found. It seems as though these monstrosities were only just able to retain their hold on life and that some inhibitory factors were present as a result of which growth was prevented. It is, however, interesting to consider an implication of this finding. It has often been thought that these doubled forms were developed from eggs that were themselves monstrous and had undergone incomplete fission at some early stage of development. Indeed " hexacanth " embryos with more than the normal quota of embryonic hooks have been described. If, however, that is true in the case of a coenurus-producing species, then we could reasonably expect that all the scolices growing in the coenurus would show the same abnormality. In this case, however, there must have been three types of scolex in one single intact coenurus. We are driven to the conclusion that the incomplete fission came early in the development of the scolex and not in that of the embryo. Perhaps, as we have seen earlier in this article, as scolices tend to be developed in groups, there may have been one or more localised germinative areas in which abnormal or incomplete fission had occurred. In this particular case there must have been at least two such areas as there were two types of abnormality in the resulting infection.

Summary.

The structure of the normal coenurus of *M. glomeratus* is described and some observations have been made on its powers of budding and vegetative growth and reproduction. It is suggested that only a small area of the cyst has the power of growth and development and that budding and daughter cyst formation is an action which attempts to stave off old age and sterility. It has been found possible to cause new growths and infections by the transplantation of certain elements to new hosts. Some remarks are made on the occurrence of abnormalities in a coenurus cyst.

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The Occurrence of Zinc and Other Metals in the Intestines of *Strongylus* spp.

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Introduction.

The actual diet of parasitic nematodes is in many cases completely unknown. As a rule, the nature of the diet has been inferred from the situation in which the worms have been found rather than by the actual examination of the gut contents. Several investigators, however, have sought haemoglobin or its products in the intestines of parasites and as a result it has been found that many forms including *Ascaris*, *Strongylus*, *Camallanus*, *Ancylostoma* and *Trichuris* have reddish-brown, weakly bi-refringent sphaerocrystals in the gut. Askanzy (1896), Looss (1905) and Fauré-Fremiet (1913) concluded that these crystals were the result of haemoglobin resorption but Von Kemnitz (1912) considered them to be zymogen granules and Quack (1913) identified them as gypsum ($CaSO_4.2H_2O$). Chitwood and Chitwood (1938) supported this view but found that some compound containing iron was present as an adsorption in the sphaerocrystals.

As the nature of the material in the intestines is important in determining the pathological effects of the parasites on the host, an attempt has been made to ascertain the exact composition of the more plentiful substances present. Following this, the host's tissues, taken from the sites where the worms were found, were analysed to discover whether the constituents recorded as occurring in the worms' gut could be obtained from the host. Those substances found both in the worm and the host were estimated quantitatively in order to determine the amount of host tissue necessary to provide the quantities present in the worm.

The difficulty experienced in separating the intestinal contents from the intestinal walls made it necessary to analyse the gut and its contents as a whole. It has been possible, however, to obtain small quantities of gut

contents free from gut wall and qualitative tests have been used to determine what substances were free in the intestinal cavity and what could be attributed to the actual worm tissue.

Preliminary Investigation.

A number of fresh specimens of *Strongylus edentatus* were taken and washed in physiological saline. The heads and tails were then cut from the worms and the intestines withdrawn by gentle traction at the anterior end. To remove traces of body fluid the intestines were quickly washed in distilled water. They were then heated in a little 10% KOH to remove the worms' tissues and oxyhaemoglobin and haematin found to be present in the intestinal contents. After centrifuging, the supernatant fluid was pipetted off and the residue rewashed in turn with KOH and water. The residue, which was a dark chocolate brown in colour, was then dissolved in a little concentrated HCl. Sulphuretted hydrogen was evolved. The solution was then diluted and passed through the ordinary group analysis for metallic radicles. A light brown precipitate was produced with H_2S in acid solution and $(NH_4)_2S$ gave a dark green precipitate in alkaline solution. Tests for the other groups gave negative results.

The Estimation of the Sulphide.

A known number of *Strongylus edentatus* females were washed and dried by gently rolling them on filter paper and weighed. The intestines were then removed and treated with KOH as explained before. The residue was dried to constant weight. To this residue about 0·3 mls. of concentrated HCl was added and the evolved H_2S passed through water and then collected in 20 mls. of N/50 I_2 solution. After all the H_2S was freed from the solution in the HCl and the water by heating, the I_2 solution was titrated against sodium thiosulphate previously standardised with potassium biiodate solution. Calculation gave the amount of sulphide recovered from the extract from the worms' gut. The results are shown in Table I.

Identification and Estimation of the Group IV Metals.

The dark green precipitate produced by the addition of $(NH_4)_2S$ to the gut extract in alkaline solution was filtered and the residue washed with dilute acetic acid. Nothing appeared to dissolve and the solution gave negative tests for manganese. However, a large proportion of the residue was soluble in 0·5N. HCl. On the addition of NaOH to this solution a

white precipitate formed which was dissolved in excess of the reagent. Sulphuretted hydrogen was then passed and a white gelatinous precipitate was formed. This precipitate was concentrated, washed and dissolved in HCl. After boiling, a little of this solution and a few drops of diphenyl-thiocarbazone in CCl_4 was added to 0·5 mls. of 2N. NaOH. On shaking, the aqueous phase was coloured red, confirming the presence of zinc.

The remainder of the residue insoluble in 0·5N. HCl was completely dissolved in fairly concentrated HNO_3. This solution gave positive tests for iron with NH_4CNS. As the residue before dissolving in HNO_3 was very small no further tests for other metals of group IV were carried out.

When quantitative results were required, a known number of *Strongylus edentatus* females were taken and washed, dried and weighed as before. The intestines were then extracted and treated as described previously. The sulphides precipitated in group IV were collected in a weighed Gooch crucible, washed with dilute $(NH_4)_2S$, dried and weighed. The ZnS was then dissolved from the contents of the crucible with 0·5N. HCl and the zinc estimated with 8-hydroxy quinoline in the normal manner (B.D.H., 1939, page 68).

The residue in the Gooch crucible, which was considered to be largely if not wholly iron sulphide, was dried and weighed in the crucible. Calculation then gave the amount of iron and zinc found in the intestines of the worms.

On one occasion phosphate was found to be present in the residue of the gut contents after extraction with KOH. One thirtieth of the solution of this residue in HCl was taken and the iron estimated by Wong's (1928) method. Calculation then gave the total amount of iron present. The phosphate was then extracted by the basic acetate method and the group tests applied in the normal manner.

The Identification and Estimation of the Group II Metals.

The dark brown precipitate produced by H_2S in acid solution was washed, dried and weighed. The amounts of precipitate recorded were so small (usually less than 1 mg.) that the normal group analysis was abandoned and spot tests were used in an attempt to identify the metals present. In view of the fact that the sulphide was brown, the tests for the following metals were carried out : lead (with diphenyl-thiocarbazone), bismuth (with acidified cinchonine and KI), tin (with dimethylglyoxime and $FeCl_3$) and copper (with potassium ferrocyanide). All these tests

gave negative results but a solution of the group II precipitate was found to oxidise benzidine to a blue meriquinoidal product. In view of the difficulty of carrying out chemical analysis on such small quantities a sample was submitted to spectrographic analysis.* Results showed that the precipitate consisted of copper with a trace of silver. Since copper was also found in the mucosa of horses' intestines it is considered that copper formed a large proportion of the precipitates obtained from other lots of worms, in spite of the negative tests obtained with potassium ferrocyanide. No doubt the group I precipitate was so small that it was not detected and was centrifuged down when the sulphide was collected.

The last entry under " Group II " in Table I represents the sample submitted for spectrographic analysis. The figure recorded is the composite weight of the CuS and AgCl.

Table I.

Average weight of worms.	Average weight of residue after KOH extraction.	No. of worms used.	Average amount of zinc per worm.	Average amount of "Group II" per worm	Average amount of iron per worm.	Average amount of sulphur per worm.
Qualitative only		10	positive	positive	positive	positive
75·44 mgs. (100)	0·998 mgs. (1·32)	10	0·58 mgs. (0·77)	negative	0·054 mgs. (0·07)	0·308 mgs. (0·40)
67·60 mgs. (100)	0·29 mgs. (0·43)	8	trace	trace	trace	0·122 mgs. (0·18)
72·73 mgs. (100)	0·593 mgs. (0·815)	17	0·346 mgs. (0·47)	trace	0·075 mgs. (0·10)	0·181 mgs. (0·24)
67·52 mgs. (100)	0·792 mgs. (1·17)	50	0·415 mgs. (0·61)	0·024 mgs.* (0·03)	0·146mgs. (0·20)	0·174 mgs. (0·26)

Table, showing the results of the analysis of the intestine and contents of *Strongylus edentatus*. In the last case shown on the table, phosphate was present but was not estimated. Figures shown between brackets represent the percentages of the total worm weight.

* This figure gives the weight of the mixture of CuS and AgCl.

The study of the intestinal contents of *Strongylus vulgaris* was carried out in a manner similar to that already described. As these worms were considerably smaller than *S. edentatus* many more worms were needed for analysis and as it was found difficult to obtain large numbers of this species

* The spectrographic analysis was carried out by Adam Hilger Ltd.

only three lots were examined. Of these, two lots were examined quantitatively. The results are shown in Table II.

Table II.

Average weight of worms.	Average weight of residue after KOH extraction.	No. of worms used.	Average amount of zinc per worm.	Average amount of "Group II" per worm	Average amount of iron per worm.	Average amount of sulphur per worm.
Qualitative only.		23	positive	negative	positive	positive
13·69 mgs. (100)	0·165 mgs. (1·20)	30	0·096 mgs. (0·70)	negative	trace	0·074 mgs. (0·54)
11·82 mgs. (100)	0·152 mgs. (1·28)	40	0·066 mgs. (0·56)	0·005 mgs.* (0·042)	0·001 mgs. (0·008)	0·058 mgs. (0·49)

Table, showing the results of the analysis of the intestinal contents of *Strongylus vulgaris*. Among the worms used in the last analysis were several males which were smaller than the females and consequently reduced the average weight per worm. Figures shown between brackets represent the percentages of the total worm weight.

* This figure shows the weight of the group II precipitate as sulphides.

Investigation of the Metals Occurring in the Intestinal Wall of the Horse.

Since *Strongylus* spp. are said to feed on the gut wall of the horse it was thought that the metals found in the worms may have been obtained in feeding and so would be present in the intestinal wall of the host. Strength was lent to this surmise by the fact that the excretion of heavy metals is carried out in the large intestine of the horse. Hence a partial analysis of horse gut wall was made. As the worms do not penetrate deeply in the hosts' intestinal walls, the mucosa from the region most favoured by the worms (a small pocket in the colon near the ilio-caecal valve) was used for analysis. This mucosa was thoroughly washed and stripped free from the muscular layers.

At first, qualitative analysis only was attempted. About 50 grms. of mucosa were prepared, dried and ashed in a porcelain crucible with concentrated H_2SO_4. The sulphated ash was then extracted with hot water which was found subsequently to contain sodium and potassium. The residue was dissolved in HCl. This solution gave positive tests for phosphate with HNO_3 and ammonium molybdate. Before eliminating the phosphate by the basic acetate method, the presence of iron was determined with NH_4CNS. The residue, after the

removal of the phosphate, contained aluminium but as the crucible had lost weight during ashing it was considered that aluminium had been obtained from it. The phosphate-free solution was then taken and passed through the group tests for metallic radicles.

Precipitates were obtained in groups II, IV, V and VI. The group VI precipitate was identified as magnesium by the use of Na_2HPO_4 and NH_4OH. Calcium, identified with excess of NH_4OH and ammonium oxalate, was found to constitute the entire group V precipitate. The sulphides obtained in alkaline solution were treated in the same manner as the similar precipitate obtained from the worms' intestines. Zinc and nickel were found to be present by the use of diphenyl-thiocarbazone and alcoholic dimethyl-glyoxine respectively. The chemical identification of the metal found in group II was difficult and its nature was finally determined in the same manner as that used in the study of the worms' intestinal contents obtained in that group. The spectroscopic examination of the one sample submitted revealed the presence of copper but no silver was found.

When quantitative results were required, the mucosa (about 150 grms. were used) was prepared as before, weighed and dried under reduced pressure at 100°C. The dry weight was determined and the substance ashed and sulphated in a weighed porcelain crucible and the weight again taken. This was then re-ashed with a known amount of fusion mixture (equal parts of Na_2CO_3 and K_2CO_3). This was washed with hot water until no sulphate could be detected in the washings. The water was then evaporated in a weighed dish and the dry weight taken. Knowing the amounts of sodium and potassium added, it was possible to find the total weight of these substances (as a mixture of sulphates) extracted from the intestinal wall.

The remainder of the ash was dissolved in HCl. This was diluted to a volume of 15 mls. and 0·10 mls. were taken and the iron estimated by Wong's (1928) method. Calculation gave the total iron present (see Table III).

The phosphate was then removed as before. The aluminium in the residue after phosphate elimination was not estimated. The phosphate-free solution was then passed through the group tests. The group II precipitate was collected by centrifuging, washed, dried and weighed as the sulphide. After filtering from the solution, the ZnS was dissolved from the group IV precipitate with 0·5N. HCl and the zinc estimated

with 8-hydroxy quinoline. Calcium was estimated as the oxalate and magnesium as the 8-hydroxy quinolinate (B.D.H., 1939, page 67).

As it was possible that not all the aluminium found during analysis had been derived from the crucible, a second analysis was made using a nickel crucible. The ash was not sulphated and it is possible that some carbon remained. The analysis was carried out as before but it was

TABLE III.

Wet wt. grms.	Dry wt. grms.	Ash. grms.	Phosphate mgs.	Iron. mgs.	Nickel mgs.	Calcium. mgs.	Zinc. mgs.	Aluminium mgs.	Magnesium. mgs.	Na_2SO_4 K_2SO_4 grms.	"Group II." mgs.
Qual. only.	—	sulp.	pos.	pos.	pos.	pos.	pos.	?	pos.	pos.	pos.
100·20 (100) Porcelain crucible	12·500 (12·3)	0·8576 (0·84) sulp.	pos.	4·14 (0·004)	neg.	?	9·05 (0·009)	?	13·5 (0·013)	0·5276 (0·506)	1·3* (0·003)
187·00 (100) Ni crucible	25·501 (13·6)	2·1732 (1·16) non-sulp.	pos.	10·5 (0·005)	?	15·2 (0·008)	5·05 (0·003)	0·94 (0·0005)	9·85 (0·005)	1·4958 (0·80)	6·4* (0·003)

A trace of chromium was detected in the last case. It is thought that this was probably obtained from the nickel crucible. Figures shown between brackets give the percentages of the net weight, The weights of the substances found do not equal the weight of ash recorded. It is thought that this is due to the fact that ashing in the nickel crucible was not complete and also because the large quantity of phosphate present was not estimated.

* These figures give the weights of the group II precipitates as sulphides.

necessary to extend the technique somewhat. Thus the sodium and potassium were sulphated during evaporation. The precipitate formed during the phosphate elimination was suspended in water and boiled with Na_2O_2 and filtered. On concentration, the solution thus obtained was a faint yellow in colour, indicating a trace of chromium. The aluminium present in this solution was estimated with 8-hydroxy quinoline (B.D.H., 1939, page 68). The presence of fairly large amounts of nickel derived from the crucible complicated the process of estimating the zinc. It was found that some of the nickel sulphide was dissolved when the group IV precipitate was washed with 0·5N. HCl. and as nickel salts interfere when estimating zinc with 8-hydroxy quinoline it was necessary to remove it before proceeding further. Therefore NH_4Cl. and NH_4OH was added

until alkalinity was reached and the nickel was then precipitated from the boiling solution with 1% alcoholic dimethyl-glyoxine. This precipitate was filtered off and the zinc estimated as before. The results of this analysis are shown in the last entry of Table III.

Situation of the Metals Recorded.

Extreme difficulty was experienced in obtaining gut contents in reasonable amounts free from intestinal wall. By passing water along the lumen of the intestine by means of a fine Pasteur pipette it was possible to obtain very small black masses of ingesta. On several occasions the material thus collected gave positive tests for zinc with diphenyl-thiocarbazone in CCl_4. It cannot be stated definitely, however, that the material examined was absolutely free from gut-wall for in dissecting out the intestine a number of cells would certainly be crushed and broken free from the gut proper.

It was thought (see discussion) that some proportion of the metals recorded were present as sulphides and, in consequence, it was considered that the irregular blackish masses found, on microscopic examination of sections of *S. edentatus*, in the lumen of the posterior parts of the intestine, might have represented metallic sulphides. A large proportion of this material, however, was probably haematin derived from the ingested hosts' haemoglobin (Rogers, 1940) being soluble in 10% KOH.

Further microscopic examination showed that the intestinal wall, throughout the major part of its length, contained small reddish-brown bodies about 4μ in diameter. These particles, which were densely massed just below the subbacillary layer rapidly becoming less numerous deeper in the intestinal wall, were probably the " sphaerocrystals " referred to in the introduction. Attempts were made to study the nature of these bodies by microscopic observation of their reactions to various chemicals. The particles were prepared in sections which had been cut in wax and the wax removed with xylol. It was found that alcohol, ether, dioxan, KOH, H_2SO_4 and HNO_3 failed to dissolve the particles, even when the latter solutions were concentrated. Hot concentrated HCl, however, dissolved the particles. Since the sphaerocrystals did not dissolve in hot 10% KOH it appears that they were among the material examined in the chemical analysis of the parasites' intestines. Though small, the particles were very numerous and may have formed an appreciable part of the weight of the intestinal material after extraction with KOH.

Since no great discrepancies between the totals of the weights of the materials estimated and the total weights before analysis were found (see Tables I and II) it appears likely that these sphaerocrystals contributed towards the metals or sulphur found. Strength is given to this surmise by the fact that the sphaerocrystals were found to withstand moderate heat, for subjection to a small flame until the tissues in the sections were charred did not appear to affect the particles greatly.

It is possible, of course, that the zinc may have been in the intestinal contents (obtained from the ingested host tissue in the process of digestion and absorption) and also in the intestinal wall. Further research is to be carried out in the matter.

Discussion.

It is not surprising that the metals, in the quantities shown in Table III, occur in the mucosa of the large intestine of the horse, since the colon of that animal is regarded as the seat of excretion of many heavy metals. Nor are the types of metals found peculiar. Todd and his co-workers (1934) have shown zinc to be necessary for the normal growth of rats and Hart, Elverhjem and Have (1937) have demonstrated that this metal was important for successful intestinal absorption and postulated a relationship between zinc and pituitary function. Wright and Papish (1929) found milk to contain zinc.

The amounts of zinc found in the horse would vary with the amounts taken in with the diet and, in consequence, the amounts found in the parasites would also vary. Drinking water would form the chief source of zinc, the amounts present depending on the source of the water and on the nature of the pipes and containers in which the water was conveyed or stored. Water passing through galvanised iron or brass pipes may contain as much as 50 parts per million of zinc, especially if the water contains much CO_2 (Drinker and Fairhall, 1933). It is thus conceivable that the amounts of zinc found in the worms would depend largely on the nature of the water taken in by the horse, great variations in the zinc content of the dry matter eaten by the horse being unlikely.

Knowing the amounts of zinc occurring in the parasites' intestines and the amount in the horse's intestinal mucosa it is possible to compute the amount of host tissue necessary to provide the zinc found in the parasite. Unfortunately, the figures shown in Tables I and II are not the results of analysis of worms taken from the mucosa of the intestines analysed to

give the figures shown in Table III. However, it seems unlikely that the relationship between the zinc in the worms and the zinc in the host mucosa would be very close, for no doubt the zinc content of different parts of the mucosa of the colon of a horse would vary somewhat and thus the zinc in the parasites would vary with their situation in the host. Therefore, it seems reasonable to use the Tables I, II and III to obtain the approximate maximum and minimum values for the amount of horse mucosa ingested.

Calculation shows, in the case of *S. edentatus*, that the parasites must ingest amounts of intestinal mucosa ranging from 3·9 to 21·2 grms. per worm to provide the zinc recorded. *S. vulgaris* would need from 0·7 to 3·4 grms. per worm. It is interesting to note the similarity in these ranges if the amounts of host mucosa ingested are given as multiples of the worm weight. For *S. edentatus* the range is from 53 to 282 times the worm weight and, in the case of *S. vulgaris*, from 62 to 248. If the zinc found in the worms were situated entirely in the intestinal lumen, these figures seem very high, for unless the zinc was being retained mechanically in the intestine, the quantities of host tissue calculated would be necessary to fill the gut only. Other alternatives are that either the parasites have a selective action in obtaining zinc and thus take it only from localities where the zinc content is much higher than as shown in Table III, or else the worm is absorbing the zinc and storing it in the gut wall. Since microscopic examination does not support the suggestion that the zinc is held mechanically in the intestinal lumen, the latter conclusion seems the most reasonable. The above figures would give, therefore, the amounts of host mucosa ingested during the life period of the worms examined. It is possible, then, that the amount of zinc present in the worms is roughly proportional to the age of the worms and, in this connection, it is interesting to note that the parasites containing the most zinc were, on the average, almost 10 mgs. heavier than the parasites containing least zinc (see Table I). In the case of *S. vulgaris* (see Table II) the larger worms also contained a higher percentage of zinc. The age factor would be influenced, of course, by the amount of zinc taken in by the horse, as mentioned before.

As the longevity of the species of *Strongylus* examined is unknown, it is difficult to assess the rate at which the host mucosa is destroyed. From analogy of cases of Ancylostomiasis and Trichostrongyliasis it appears that the life of the majority of adult *Strongylus* spp. may be about

12 months (it must be emphasised that this is purely a hypothetical suggestion) in which case 100 *S. edentatus* would destroy from 400 to 2,100 grms. of mucosa per year. These figures may be low for it is not likely that all the zinc taken in with the ingested mucosa is retained. When the blood loss caused by the parasites (Rogers, 1940) is considered it is apparent that horse Strongyles may cause considerable loss in condition for heavy infestations are not uncommon.

The other metals found in the horse mucosa and in the parasites do not seem unusual. Aluminium was found by Myers and Morrison (1928) in the tissues and blood of dogs. Rusoff and Gaddum (1938) found aluminium and copper to be present in newborn rats. No doubt most of the metals recorded in the host but not in the worm were removed by the treatment with KOH. The parasites' intestines were not ashed in the normal manner, for the estimation of the sulphide present was considered to be of some importance (see later). The iron recorded from the host was probably largely obtained from haemoglobin but that in the worm, though it may have been ingested as haemoglobin originally, was probably from simpler compounds, the haematin present being extracted with the KOH (Rogers, 1940). Silver has been found in animal tissue on several occasions.

Since H_2S was evolved when the residue obtained after extraction of the worm intestines with KOH was treated with HCl, it appears that a metallic sulphide was present. The proportions of residue, zinc and sulphur shown in Tables I and II have been retabulated in Table IV, taking the amounts of zinc present as unity.

TABLE IV.

Residue after extraction with KOH.	Zinc.	Sulphur.
1·7	1·0	0·51
1·8	1·0	0·51
1·9	1·0	0·43
1·8	1·0	0·80
2·3	1·0	0·88

Table showing the proportions of residue, sulphur and zinc (taken as unity), found in the worms' intestines.

The first three cases shown are taken from Table I, the last two from Table II.

Since the theoretical ratio of zinc to sulphur in ZnS is 1 : 0·49, it appears possible that, in the case of *S. edentatus*, this compound was present. It is unlikely that ZnS could be tolerated as such in living tissue and if present in the gut wall it may be there in an organic compound in which the zinc and sulphur are held in equiatomic proportions. In *S. vulgaris* the proportion of atoms of zinc to atoms of sulphur may be 2 : 1. Thus it may be thought that the sphaerocrystals are a compound, which, when treated with KOH give the ZnS which may have been present in the residue. Experimental observations, however, do not support this hypothesis for, after treating the sphaerocrystals with KOH, no bubbles of gas were seen when HCl was applied. Further investigation is necessary and the possibility that zinc, or a zinc-containing compound, is acting as a sulphur acceptor in sulphur metabolism must not be overlooked.

The proportion of zinc to the residue (see Table IV) is remarkably constant. This would be expected if zinc and sulphur were always present in a fixed relationship and together formed a large proportion of the residue.

The $CaSO_4.2H_2O$ which Chitwood and Chitwood (1938) suggested may form the sphaerocrystals has not been detected. As it is not known what species were actually used in that investigation, little comment can be offered. However, under the conditions used by Chitwood and Chitwood (the crystals were formed in concentrated HCl) $CaSO_4.2H_2O$ would probably form monoclinic crystals and ZnCl regular crystals and it is unlikely that the two would be confused.

Summary.

1. The intestines and their contents taken from several lots of *Strongylus edentatus* and *S. vulgaris* have been analysed and the amounts of certain metals and sulphur present have been estimated.

2. Zinc was found to be the most plentiful metal present, reaching a maximum of 0·58 mgs. per worm. Copper, silver and iron were the other metals recorded.

3. The amounts of sulphur estimated indicate that the zinc was probably present as the sulphide. The possible situation of the zinc and sulphur-containing compound is discussed.

4. Partial analysis of the mucosa of the horse taken from regions where the parasites were most frequently found revealed the presence of zinc and copper. The zinc reached a maximum concentration of 9·05 mgs.

TABLE V.

Percentage of Dry Matter in Sheep's Faeces.

Day	Sheep I	Sheep II	Remarks
1	19·8	20·3	Normal
2	21·2	21·1	Normal
3	23·7	22·2	Dryish pellets
4	19·0	17·4	Mushy

TABLE VI.

Analyses of Variance : Wet and Dry Faeces.

Wet Faeces.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Variance	Variance Ratio
Sheep	231·889	1	231·889	56·47
Days	225·341	6	37·557	9·15
Lots	285·92	8	35·740	8·70
Series	743·15	15	—	—
Techniques ...	53·24	1	53·24	14·27
Interaction ...	55·98	15	3·732	—
Batches	852·37	31	—	—
Error	657·05	160	4·1066	—
Total	1509·42	191	7·903	—

Dry Faeces.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Variance	Variance Ratio
Sheep	5813·92	1	5813·92	60·01
Days	3970·98	6	661·83	6·83
Lots	6066·47	8	758·31	7·83
Series	15851·37	15	—	—
Techniques ...	1247·58	1	1247·58	14·36
Interaction ...	1303·21	15	86·88	
Batches	18402·16	31	—	
Error	15502·17	160	96·888	—
Total	33904·33	191	177·51	—

root of the error variance as our standard deviation in testing for significant differences between the various means for days, lots, and techniques.

We mentioned above that the variance for counters was trivial and would not be further considered. As this is quite an unorthodox procedure and would probably be frowned upon in the best statistical circles, it may be as well to state that the sum of squares (and also the variance) for counters was 0·3654, a value negligible in size but not significantly smaller than the corresponding interaction variance even at the 20% point and therefore not suspiciously small.

We shall now compare the various means. The standard deviation will be the same for all comparisons on a wet basis (s=2·027). Theoretically the dry-basis value should be the same, since the variation among successive counts from the same suspension is indifferent to the dryness of the faeces, but we shall use the calculated value : s=2·015. The critical difference at the 5% point, differences greater than which will be judged significant, will vary according to the number of values on which each mean is based. In general, the critical difference is given by :

$$t\sqrt{2s^2/n},$$

where s^2 is the error variance, n is the number of values contributing to the mean, and t is the tabulated 5% value of t for the number of degrees of freedom on which the error variance is based. In our case t is taken as 2, and the critical differences will be :

Day means : (n=24). Wet counts : 1·1700 ; Dry counts : 1·1639.
Lot means : (n=12). Wet counts : 1·6547 ; Dry counts : 1·6459.

Days.—The two sheep obviously have a different level of infestation which will not be further considered. The day means in order of magnitude, for wet and dry faeces and for each sheep separately, are in Table VII. In the column of differences significant differences are in italic type and a bracket means that the included differences achieve significance when summed. Thus, for sheep I, day 1 is higher than 4 and 2 on a wet basis, and 1 than 2 on a dry basis ; for sheep II, 3>1>4 and 2 on a wet basis, and day 3 is higher than the rest and day 2 lower than the rest on a dry basis.

From these results we may conclude that there is a real difference in the counts for any one sheep from one day to another on most days, and that such significant differences are reduced in number but not entirely obliterated by expressing the counts on a dry basis : in fact, some of the

dry-count differences are exaggerated. Hence the day to day variation is only partly to be explained in terms of the water-content of the faeces.

There is a suggestion that the daily variation is not entirely independent as between the two sheep : day 1 has a high count and day 2 a low one for both sheep, on either basis. It is quite conceivable that there is

TABLE VII.

Day means in order of magnitude, with differences.

Wet Faeces.

Sheep I			Sheep II		
Days	Means	Differences	Days	Means	Differences
1	3·50		3	7·13	
		0·67			*1·87*
3	2·83	*1·17*	1	5·26	
		0·50			*1·26*
4	2·33		4	4·00	
		0·08			0·67
2	2·25		2	3·33	

Dry Faeces.

1	3·62		3	6·57	
		1·10			*1·27*
4	2·52	*1·44*	1	5·30	
		0·04			0·59
3	2·48		4	4·71	
		0·30			*1·47*
2	2·18		2	3·24	

some overriding factor causing simultaneous daily variation in all the sheep of a flock ; pasture or weather changes suggest themselves ; but, if so, our analysis of variance is not so designed as to bring out this daily trend. To do this, days should be made primary and sheep subordinate. The first two items in our analysis should be reversed in order, therefore, as in Table VIII. Here, the sum of squares for days is based on the grand daily totals and means of 48 values and has 3 degrees of freedom. The sum of squares for sheep is also appropriately adjusted and has one degree of freedom for each day. For these two factors the sums of squares and degrees of freedom will add to the same totals as before, the rest of the analysis remains the same, and the same error variance applies. All variances are again significantly high and the critical differences for means are 0·8273 (wet basis) and 0·8231 (dry basis). The four means

are compared in the lower part of Table VIII from which we may conclude that, taking both sheep together, days 3 and 1 are higher than 4 and 2 on both wet and dry bases ; in addition the dry counts show that day 4 is higher than day 2, so that here the dry counts have brought out a significant difference which would otherwise have been missed.

TABLE VIII.

Revised Analyses with Days primary to Sheep.

Wet Faeces.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Variance	Variance Ratio
Days	150·663	3	50·221	12·23
Sheep	306·567	4	76·642	18·66
Dry Faeces.				
Days	2505·22	3	835·07	8·62
Sheep	7279·68	4	1819·92	18·78

Day Means in order of magnitude (Both sheep).

Wet Faeces			Dry Faeces		
Days	Means	Differences	Days	Means	Differences.
3	4·98	0·60	3	4·53	0·07
1	4·38	*1·21*	1	4·46	*0·85*
4	3·17	0·38	4	3·61	*0·90*
2	2·79		2	2·71	

Lots.—The lot means are compared, in pairs, in Table IX which shows that there was a significant difference between the two lots of a single motion on one day out of four for each sheep, but not the same day. The dry-basis counts confirm this. The result was not really expected, since it might be thought that eggs of the trichostrongyle type would be well mixed with the faeces by the time they were passed. Evidently this is not always the case, and one must be prepared for an uneven distribution of eggs in the motion of faeces occasionally—so far as our evidence goes, on one day in four.

TABLE IX.

Lot Means in order of magnitude, with Differences.

Wet Faeces.

	Sheep I		Sheep II	
Day	Means	Differences	Means	Differences
1	4·58	*2·16*	5·67	0·84
	2·42		4·83	
2	2·58	0·66	3·50	0·33
	1·92		3·17	
3	3·42	1·17	10·25	*6·25*
	2·25		4·00	
4	2·92	1·17	4·17	0·34
	1·75		3·83	
		Dry Faeces.		
1	4·74	*2·24*	5·72	0·84
	2·50		4·88	
2	2·50	0·64	3·40	0·32
	1·86		3·08	
3	3·03	1·09	9·46	*5·77*
	1·94		3·69	
4	3·15	1·26	4·90	0·39
	1·89		4·51	

Techniques.—The McMaster method, as modified to suit this experiment, gives significantly higher counts than the modified Stoll method, the differences being 1·0521 for wet counts and 1·0465 for dry, both relative to a general mean of 3·828 (see Table X). These differences have high significance since they would arise by mere chance only once in over 1,000 similar trials. It does not necessarily follow that the McMaster counts are the more accurate. As already explained, the eggs from 1 gm. of faeces were concentrated by sugar-flotation and counted, in the case of one of the two lots of faeces from each sheep on each day, giving 8

counts in all. These counts were divided by 200 to make them comparable with the other two sets and the error variance was determined, excluding the variance due to differences between the two sheep (the

TABLE X.

Comparison of " Stoll " and " McMaster " Techniques.

Technique	Mean	Variance	Standard Deviation	Variability
" Stoll "... ...	3·3021	4·3468	2·085	63·18 per cent.
" McMaster " ...	4·3542	3·8664	1·966	45·09 per cent.
Difference ...	1·0521	0·4804	—	18·09
Standard Error of Difference	0·2925	0·9257	—	5·602

other extraneous factors cannot be excluded in this case). The mean of the 8 counts was 3·377, a value almost midway between the Stoll and McMaster means which were 2·938 and 3·711 respectively, and the error variance was 0·6978. From the added sums of squares and degrees of freedom for error belonging to the Sugar and Stoll, and to the Sugar and McMaster distributions, respectively, two standard deviations were calculated, by means of which the two differences could be compared with their standard errors. Neither the Sugar-Stoll nor the Sugar-McMaster difference was significant, so that the question of accuracy cannot be decided. We can conclude that the McMaster counts are significantly higher than the Stoll, and that there is some indication of overestimation by the former and underestimation by the latter.

For the purpose of finding whether one of the two techniques was less variable than the other, the whole of the wet-basis counts were used giving in each case 80 degrees of freedom for the error variance. The relevant data are set out in Table X. The standard error of the difference between the two variances was taken as :

$$\sqrt{2\ (s_1^4 - s_2^4)/80},$$

and is actually larger than the difference. The two variances therefore do not differ significantly. Nevertheless, the coefficients of variability show that the McMaster technique is the less variable of the two.

As in part one, the regression of log (standard deviation) on log (mean) has been determined for the two techniques separately, giving the following coefficients :

Stoll, $b=0{\cdot}61637\pm0{\cdot}1400$,
McMaster, $b=0{\cdot}52020\pm0{\cdot}2299$,
(Part One, $b=0{\cdot}55845\pm0{\cdot}02089$.)

The first two have been compared with the third by calculating standard errors for the differences based on variances compounded from the appropriate pairs of variances. None of the three differences was significant, each difference being less than its standard error. So far as the differences may be considered suggestive, however, the McMaster value is the closer to the Part One value and the closest of the three to the theoretical Poisson value of 0·5. If, for the two techniques, the usual 5% error-limits are plotted for each regression line, the Poisson line lies wholly within these limits in both cases.

The McMaster technique cannot be shown to be significantly more accurate than the Stoll on the limited evidence available, but so far as it goes the evidence points in that direction. In addition, we have found that it takes about three times as long to do an equal number of Stoll counts, so that on all grounds the McMaster technique is preferable, particularly where numerous counts have to be made.

Dry-Basis Counts.—The variable amount of water in faeces from the same animal on different days, or from different animals on the same day, is obviously capable of a serious effect on egg-counts. In our own experiment, covering only two sheep for four days, this effect was not very great. Table V shows that the variation in the dry-matter percentages was quite small. The faeces from both sheep on the fourth day were rather mushy, but no extreme conditions were met with, and more extensive trials are desirable. Most of the conclusions drawn from wet-basis values were confirmed by the dry-basis ones, and we have learnt nothing from the latter that the former could not have taught. But in other trials involving greater variation in the dryness of the faeces it might be otherwise, and this factor should not be overlooked. Its neglect would not affect the error-variance but would increase the variance due to days and might increase or decrease that due to treatment or other factors. The effect on the day-variance is seen in Table VI where the variance ratios on a dry basis for days and lots were reduced and inverted

in order, compared with the wet-basis values, showing that part of the day to day variation is due to the varying amount of water in the faeces.

General Discussion.

The first part of this paper has shown that dilution-counts made by a modification of the Stoll technique are distributed in reasonably good agreement with the Poisson series, thus indicating that the technique is reasonably reliable. Half the counts in the second part were made by a modified McMaster technique, and these show (Table X) that the variance falls below theoretical equality with the mean. As in the first part, however, the discrepancy is scarcely significant so that this technique can also be considered reliable. It is now in general use at this Institute and, as counts accumulate from various experiments, it will be possible to examine more fully their agreement with the Poisson distribution.

The general thesis of this paper is that, in experiments involving dilution-counts, it is essential to deal with the problem of variation, so that the significance of treatment-differences may be accurately assessed. If this is done in a properly designed experiment far more reliable information can be extracted from it, for a given expenditure of time and materials than in a badly designed experiment where variation is ignored. The essence of trials that take account of variation is the use of the " error-variance " as a measuring stick to assess the significance of treatment or other variances. It follows, therefore, that the error variance must be kept as small as possible. The true error variance, measuring variation among successive samples from the same suspension of eggs, is fundamental : no smaller error variance is possible. But, if the experiment has been badly designed, other sources of variation may be confounded with the true error variance, thus increasing its size and thereby making it a less sensitive instrument for its purpose of assessing significance. The chief aim of the second part of this paper has been : (i) to show that some of these other sources of variation are significantly large and therefore worthy of attention, and (ii) to suggest the kind of statistical method by which such extraneous sources of variation can be separated out and excluded from the error variance. If this point requires illustration, the wet-basis analysis of variance in Table VI may be considered. Here, the total variance due to all factors is 7·903, whilst the true error variance is only 4·107 : the extraneous factors have nearly doubled the variance.

It is worth while, then, so to design the experiment and its analysis that all the extraneous sources of variation are excluded from the error variance.

Summary.

1. A set of 275 egg-counts, made by a modification of Stoll's dilution-technique and involving 11 series of 25 counts each (each series having a different mean), was found to be distributed in approximate agreement with the Poisson distribution. Although from its nature not normal in form, it showed no significant departure from the corresponding normal curve.

2. For means up to about 15, using this technique, it is safe to take the variance as numerically equal to the mean : for higher means the variance is greater than the mean.

3. An investigation (involving 192 egg-counts) into the variation of counts from day to day and from one portion of the faeces to another showed that both of these factors were significantly greater than the variation between counts from the same suspension of eggs.

4. The daily up-and-down variation is partly a factor working independently in each animal and partly an overriding factor affecting animals together. It is significant on most days, and is not nullified by expressing counts in terms of dry faeces.

5. The variation from portion to portion of a motion of faeces was found to be significant on only one day in four : this also is not eliminated by dry-basis counts.

6. The same 192 counts were used to assess the difference between two techniques : (*a*) suspending eggs in water and measuring 0·15 c.c. of the suspension with a McDonald pipette, and (*b*) suspending the eggs in a 50%-saturated salt solution and measuring 0·15 c.c. in a McMaster counting slide. As compared with the former, the latter gave significantly higher counts and showed less variability. Both methods gave a line for the regression of log s on log $\bar{x}$ wholly consistent with the expected Poisson line. In addition, counting is about three times as rapid by the McMaster

method. As judged by comparison with counts of eggs concentrated by sugar-flotation, it was not possible to decide which was the more accurate method.

7. In the present investigation, the daily variation in the amount of water in faeces was not sufficiently large to invalidate conclusions drawn from wet-basis counts.

8. The general bearing of these findings on the design of anthelmintic experiments is briefly discussed.

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Digestion in Parasitic Nematodes. 1. The Digestion of Carbohydrates.

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Introduction.

The physiological processes of digestion in nematodes have not been studied to any extent. Hoeppli (1927) found that extracts from the anterior section of *Strongylus* spp. acted on ingested epithelial cells but he did not study the actual nature of the digestive processes deeply. Some evidence has been presented by Schopfer (1932) that *Ascaris* secretes a proteolytic enzyme and Rogers (1940) has discussed the digestion of haemoglobin by *Strongylus edentatus* and *S. vulgaris*. The division of the Nematoda into forms in which the intestine both secretes and absorbs, like *Ascaris*, and forms like *Proleptus obtusus* where oesophageal glands provide the digestive enzymes and the intestine is purely absorptive has been advocated by Yonge (1937). Lapage (1937) suggests that generalizations like this cannot be made. Indeed, it appears that a complete study of digestion and absorption has not been approached in any species of nematode parasite.

Other groups of parasites have been investigated more fully but apparently with negative results. Rogers (1927) reviewing the distribution of enzymes in the animal kingdom states that pepsin, trypsin, rennin, lipase, maltase and amylase are absent in Trematodes and Cestodes.

For the purposes of this investigation *Ascaris lumbricoides* (pig strain) and *Strongylus edentatus* have been used, both forms being easily obtainable and of a convenient size. The physiology of digestion of these parasites may differ considerably for *A. lumbricoides* is found free in the anterior section of the small intestine of the pig and probably obtains most of its nourishment from partially digested matter in the host's intestine whereas *S. edentatus* remains firmly attached to the mucosa of the large intestine of the horse and appears to obtain its nutriment from the host's blood and tissue (Rogers, 1940).

It is thought that, apart from its fundamental interest, the investigation of the digestive systems of nematodes may be important in the estimation of the effects of the parasites on the host for the nature of the digestive processes would indicate the type of tissue and juices most vigorously attacked by the enzymes. Such tissues and juices probably would be those taken from the host's gut wall or intestinal contents by the parasite.

Method of Extracting the Active Enzyme.

Fresh specimens of *Strongylus edentatus* were washed rapidly in distilled water. The heads were cut off just before the most muscular regions of the oesophagus (by severing the alimentary canals in such regions, the escape of intestinal contents was reduced to a minimum). After removing the tails from the worms the intestines were withdrawn from the bodies of the parasites by gentle traction at the anterior ends and washed to remove traces of body fluid. The oesophagus was then severed from each intestine and the intestines separated into equal anterior and posterior sections. The three lots of material thus obtained were then separately ground to a fine emulsion with washed sand in a little 50% glycerol. The enzyme preparations were centrifuged to remove the sand and any coarse material. As a rule, about 2 ml. of anterior intestinal preparation, 2 ml. of posterior intestinal preparation and 0·5 ml. of oesophageal extract were formed from 150 worms. For each 2 ml. of preparation 1 drop of 5% thymol in alcohol was added.

Extracts of the whole alimentary canals of the worms were used also. For some purposes watery extracts were prepared. In the case of *Ascaris lumbricoides* glycerol and water extracts were made in a similar manner, 150 worms giving about 20 ml. of enzyme preparation.

General Method of Amylolytic Experiments.

The reactions were carried out in small test tubes (10 mm. by 60 mm.). As a rule, 0·8 ml. of solution containing 0·1 ml. of 2% solution of soluble starch and 0·2 ml. of glycerol enzyme extract was used. The tubes were incubated at 37°C. and at regular intervals 0·03 ml. of the test mixture was withdrawn and mixed with an equal quantity of dilute acidified iodine solution on a spotting plate. When almost all the starch in the test mixture had been broken down to form sugar and erythrodextrin under the action of the enzyme, the addition of iodine solution gave a reddish colour to the mixture. A coloured chip was used for comparison in obtaining the standard red colour. In estimating the relative enzyme

activity over a range of conditions the times taken for the digestion of the starch to reach the erythrodextrin stage were noted. The reciprocals of the times taken to reach this arbitrary end point are regarded as representing the velocity of action and, when expressed as percentages of the fastest rate in series of experiments, will be termed " relative velocity."

When an optimum of a series of conditions was required, tubes were set up, incubated at 37°C. and samples from all tubes were examined with iodine on the spotting plate after the same period of incubation. The reacting mixture showing the greatest change was considered to represent the optimum condition. Blue, purple, red or colourless solutions were obtained depending on the presence of starch, erythrodextrin or achroodextrin, the latter two substances being formed in turn by the digestion of the starch.

Effects of Salts on Amyloclastic Activity.

(*a*) *Strongylus edentatus*.—The actions of the following salts were examined: KI, NaCl, Na_2HPO_4, Na_2SO_4 and $NaNO_3$. A control tube in which distilled water was added instead of salt was also used.

Tubes containing 0·5 ml. of salt (0·5M solution), 0·1 ml. of anterior worm gut glycerol extract, and 0·1 ml. of starch (a 2% solution) were incubated at 37°C. The times of reaction were taken by examining the samples from the tubes at regular intervals. After 30 minutes the order of activity could be detected. Digestion had proceeded the farthest in the tube containing KI, Na_2HPO_4 next, and the tubes containing Na_2SO_4 and NaCl next. In the control and $NaNO_3$ tubes no changes could be detected.

The results showing the times necessary for digestion to reach the arbitrary end point are shown in Table I.

Salt.	Relative amyloclastic activity.
Control (none added)	9
Sodium nitrate	11
Sodium chloride	25
Sodium sulphate	30
Disodium hydrogen phosphate	33
Potassium iodide	100

Table I showing the effects of salts on the rapidity of carbohydrate digestion by the glycerol extract of the gut of *Strongylus edentatus*.

(*b*) *Ascaris lumbricoides*.—The actions of the following salts were examined : $NaHCO_3$, Na_2HPO_4, NaCl, Na_2SO_4, and KI.

Tubes containing 0·5 ml. of whole gut glycerol extract, 0·5 ml. of starch (2% solution) and 0·5 ml. of salt (0·5M solution) were incubated at 37°C. Samples were withdrawn and examined as before. After 20 minutes incubation, the salt giving optimum conditions for digestion could be detected as mixtures with iodine solution gave the following results : $NaHCO_3$, colourless ; Na_2HPO_4, pink ; Na_2SO_4, reddish-purple ; NaCl, purple ; Kl and the control, blue. The times taken to reach the end point were also noted. These results are summarized in Table II.

Salt.	Relative amyloclastic activity.
Control (none added)	Slow
Potassium iodide	Very slow
Sodium chloride	11
Sodium sulphate	17
Disodium hydrogen phosphate	50
Sodium bicarbonate	100

Table II showing the effects of salts on the rapidity of carbohydrate digestion by the glycerol extract of the gut of *Ascaris lumbricoides*. Digestion in the control and KI tubes was very slow and the figures have not been included in the table.

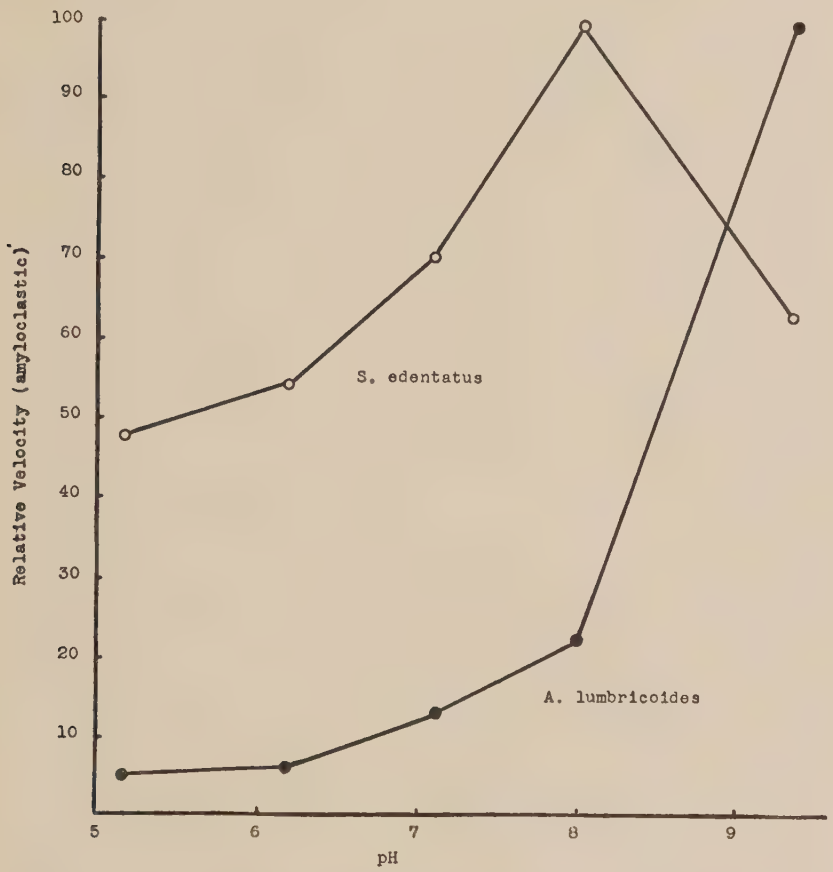
Effect of Hydrogen ion Concentration on Amyloclastic Activity.

Strongylus edentatus and *Ascaris lumbricoides*.—Amyloclastic action was examined at *p*H 5·3, 6·2, 7·1, 8·0 (phosphate buffers) and 9·4 (bicarbonate-phosphate buffer). Tubes containing 0·5 ml. of buffer, 0·1 ml. of starch (2% solution) plus 0·2 ml. of anterior gut glycerol extract in the case of *S. edentatus* or 0·5 ml. of whole gut glycerol extract in the case of *A. lumbricoides* were set up and incubated at 37°C. At regular intervals the progress of digestion was examined by the use of iodine as described before. The times taken to reach the end points were noted and the results are shown in Graph I.

Situation of the Amylolytic Enzyme in *S. edentatus*.

Amyloclastic activity of glycerol extracts from the anterior, posterior and oesophageal regions of the alimentary tract of *S. edentatus* were compared. True comparison was difficult for the sections of the gut used in preparing the extracts could not have been of equal size and no

doubt enzyme was present unevenly distributed along the lumen of the intestine. This trouble was overcome to some extent by extracting the active agent with amounts of 50% glycerol proportionate to the weights



Graph I showing the effects of hydrogen ion concentration on the relative velocity of the amyloclastic action of amylases from the intestines of *Strongylus edentatus* and *Ascaris lumbricoides*.

of the different sections of the alimentary tract. The activity of the extractives from the three regions was tested at *p*H 8·0. After an hour's incubation the degree of digestion of starch was examined with iodine solution. Digestion by the oesophageal fraction was undiscernible and the activity of the posterior gut extract was somewhat less than that of the anterior gut. It is possible, of course, that the enzymes were not in

alimentary canal tissue at all and the differences in activity noted in the various regions was due to enzyme mixed with the ingesta which was most bulky in the anterior gut section.

General Method of Saccharogenic Experiments.

In these experiments the amounts of sugar produced by the digestion were measured. Test tubes containing buffer, enzyme and starch were incubated at 37°C. for a given period and then the sugar produced was estimated by Somogyi's (1926) micro modification of the Shaffer and Hartmann method of determining blood sugar with reagent No. 1. The buffers were similar to those used in the amyloclastic experiments. Tests carried out on blanks showed that the amount of phosphate present did not appreciably affect the determination of the sugars (cf. Visscher, 1926).

The approximation that the hydrolysis of starch is a linear function of time has been assumed to be accurate enough for the purposes of estimating reaction velocities. These, therefore, have been considered to be proportional to the amounts of sugar produced in a given time.

(*a*) *Strongylus edentatus*.—Test tubes containing 0·2 ml. of anterior gut glycerol extract, 0·5 ml. of buffer and 0·5 ml. of 2% starch solution were incubated for 17 hours after which the reducing sugar present in the different tubes was estimated. The amounts found are shown in Table III and reaction velocities are compared in Graph II.

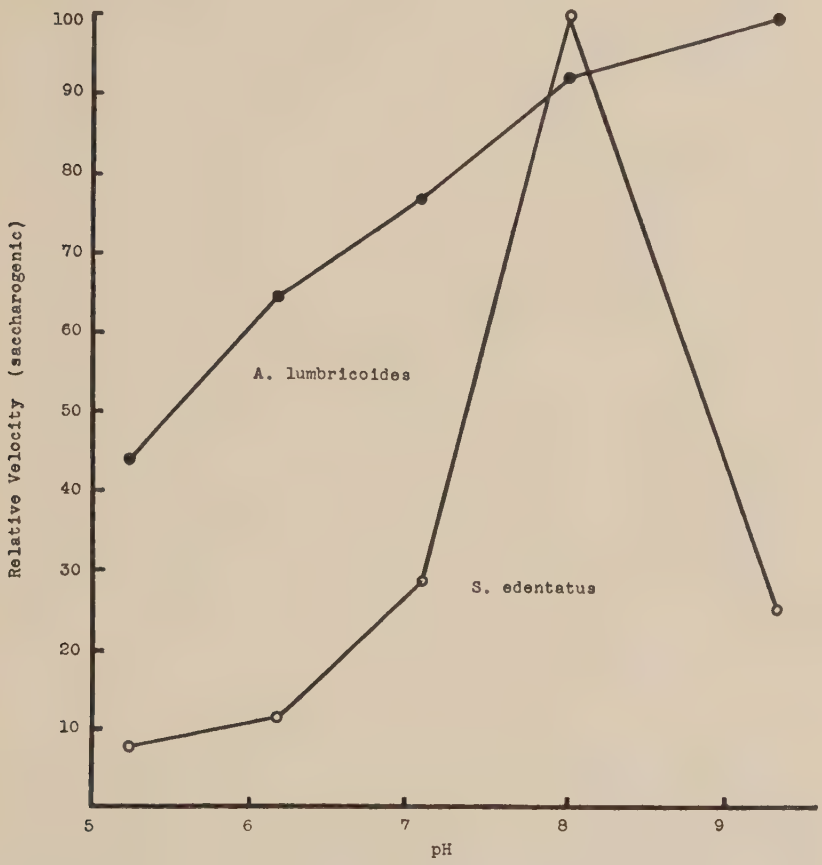
Similar experiments were carried out using whole gut and posterior gut extracts. In every case, a similar type of curve was obtained, maximum activity occurring at *p*H 8·0.

	Reducing sugar (mgs.).	
*p*H	*S. edentatus.*	*A. lumbricoides.*
5·3 ...	0·01 ...	0·38
6·2 ...	0·14 ...	0·56
7·1 ...	0·37 ...	0·66
8·0 ...	1·28 ...	0·80
9·4 ...	0·30 ...	0·86

Table III showing the production of reducing sugar by the digestion of starch by *S. edentatus* and *A. lumbricoides*. Figures for the amounts produced by the two parasites are not strictly comparable.

(*b*) *Ascaris lumbricoides*.—Test tubes containing 0·5 ml. of buffer, 0·5 ml. of whole gut glycerol extract and 0·5 ml. of 2% starch solution

were incubated for 15 hours and then examined for reducing sugar as before. The results are shown in Table III and Graph II.



Graph II showing the effects of hydrogen ion concentration on the relative velocity of the saccharogenic action of amylases from the intestines of *Strongylus edentatus* and *Ascaris lumbricoides*.

Invertase Activity.

The hydrolysis of 5% sucrose solution by the enzymes extracted from *S. edentatus* and *A. lumbricoides* was studied. The amounts of reducing sugar formed in a given time were estimated as explained previously.

Preliminary investigation showed, that in both parasites, invertase activity was so slight as to render detailed study unnecessary for the

purposes of this research. Activity of water extracts of whole *A. lumbricoides* gut in relation to hydrogen ion concentration was slight from *p*H 5·3 to *p*H 9·4. In the case of *S. edentatus*, the effect of glycerol, which inhibits the hydrolysis of sucrose by yeast invertase, was studied. It did not appear to be important for the production of reducing sugar in solutions buffered at *p*H 8·0 containing 5% and 27% glycerol respectively, was similar. Even after 24 hours' incubation the amounts of reducing sugar present were small. Nor did it appear that hydrogen ion concentration was important though the activity at high concentrations (*p*H 5·3) was greater than at low concentrations (*p*H 9·4).

The Nature of the Sugars Formed During Digestion.

After incubation for 24 hours at *p*H 8·0, the products of the digestion of starch by *S. edentatus* whole gut extract were examined as osazones, the digested material plus phenylhydrazine hydrochloride and sodium acetate being heated in a boiling water bath for 45 minutes, slowly cooled and then examined microscopically. Dextrosazone crystals could be seen. Evidently, the digestion of starch by *S. edentatus* proceeds by the splitting off of dextrose molecules.

Carbohydrate digestion by *A. lumbricoides* was similarly examined. Even after repeated attempts, however, no definitely recognisable crystals could be found, though small yellow globular bodies were universally present in the digested material after treatment with the phenylhydrazine hydrochloride.

Discussion.

Clearly the amylolytic enzymes present in *S. edentatus* and *A. lumbricoides* differ widely. In the case of the former, KI activated the amyloclastic process greatly, whereas it gave no assistance to the action of the latter. Sodium chloride was not an efficient activator for the parasite amylases and it is surprising that Na_2SO_4 and Na_2HPO_4 should have been of greater assistance. These results are in strong contrast to those obtained by Wigglesworth (1927) with cockroach amylase and ptyalin when NaCl was found to give maximum activity and KI was the third most active salt. Sodium dihydrogen phosphate and Na_2SO_4 had little influence on the activity of these enzymes. It appears, therefore, that the *S. edentatus* and *A. lumbricoides* amylases differ from one another and also from the cockroach amylase and ptyalin in the way they are influenced by salts.

The effect of hydrogen ion concentration on the activity of *S. edentatus* amylase was most marked (see Graphs I and II) activity reaching a maximum at *p*H 8·0. Since the relative velocity of reaction at *p*H 8·0 was more than 30% faster than at the other hydrogen ion concentrations examined, it appears that the range of *p*H in the intestines of the worms would probably be fairly small. In this connection, it may be noted that Rogers (unpublished) has found that the average *p*H of the intestinal contents of 6 lots of *S. edentatus*, was 7·82, the limiting values being *p*H 8·03 and *p*H 7·71.

In the case of *A. lumbricoides* amylase, activity reached a maximum over the range of *p*H examined, at 9·4. It has been shown (see Table II) that $NaHCO_3$ strongly activated the amylase from this parasite and as bicarbonate was present at *p*H 9·4 only (borate buffers could not be used owing to the formation of the highly dissociated glyceroboric acid in the presence of glycerol) it may be considered that the effect of the hydrogen ions was overshadowed by the presence of the salt. However, phosphate also assists the action of the enzyme and though the difference in activity at *p*H 8·0 and *p*H 9·4 may be due, to some extent, to the different salts present, it appears that the hydrogen ion concentration has also contributed to this difference. Additional evidence to support this contention has been found in the study of the action of the lipolytic enzyme of *A. lumbricoides* which is also most active at *p*H 9·4.

It may be thought that the starch-splitting enzyme found in *A. lumbricoides* was simply the host's pancreatic amylase ingested by the parasite with the food material from the lumen of the pig's intestine. (Li (1933), Hoeppli (1927) and others maintain that *A. lumbricoides* obtains its nutriment by ingesting the partially digested food material in the host's gut.) However, the optimum hydrogen ion concentration for the action of amylase from fresh pig pancreas is *p*H 5·5 to 6 (Groll, 1924). Evidently the amylases in the parasite and the host are different enzymes.

It is apparent that the parasites examined had comparatively efficient systems for the digestion of starch. At first, this may appear surprising, especially in the case of *S. edentatus*, where the diet is probably composed of host blood and tissue entirely (Rogers, 1940 and 1940a). It must be remembered, however, that a part of the carbohydrate ingested by *Strongylus edentatus* would be glycogen and in that case amylase may be necessary to prepare monosaccharide for absorption. *A. lumbricoides*

has greater need of carbohydrate splitting enzymes for this parasite probably ingests considerable amounts of starchy material some of which, however, would be partially digested.

As it is unlikely that sucrose, maltose or lactose would be present in the diet of *S. edentatus*, the lack of powerful invertases would not be important. Since dextrose was formed from starch by the action of parasite amylase, the same monosaccharide would probably be formed from ingested glycogen. This dextrose, together with that present as such in the ingested host tissue and blood, would be the chief form of carbohydrate in the digested material in the intestines of *S. edentatus*. It seems reasonable to suggest, therefore, that carbohydrate is absorbed in the intestines of these parasites largely in the form of dextrose.

In view of the fact that the amylolytic enzymes of *S. edentatus* and *A. lumbricoides* are distinct, it seems likely that they both differ from the primitive digestive enzymes of the free-living ancestors, the specialised life assumed by the worms in becoming parasitic favouring the survival of forms with digestive systems which could function in harmony with the hosts' juices in the situations they occupied. In the case of *A. lumbricoides*, the fluid taken into the intestine from the host would often be fairly alkaline. Long and Fenger (1917) found that the reaction of the intestinal contents of hogs varied from *p*H 6·48 to *p*H 8·05. If the head of *A. lumbricoides* was buried under mucus at all, it is more likely to be influenced by the secretion of the duodenal glands, which, in the case of the pig, varies from *p*H 8·4 to *p*H 8·9 and contains a considerable amount of bicarbonate (Florey and Harding, 1934). It would thus appear that the optimum conditions for amylolytic activity in *A. lumbricoides* would not be greatly disturbed by the ingestion of fluid from the hosts' intestines.

In the case of *S. edentatus*, free intestinal contents of the horse may not be taken in by the parasite except when a new area of mucosa is attacked, at which time the layer of fluid on the mucosa under the buccal capsule would be all that could affect the nature of the material ingested. Danniger, Pfragner & Schultes (1928) found the average *p*H of the contents of the caeca of horses to be 8·12. This, combined with the host tissue and blood (*p*H 7·20–7·55, Brey, 1926) would again represent a material of optimum reaction for the activity of parasite amylase.

Summary.

1. Amylolytic enzymes have been extracted from the intestines and contents of *Strongylus edentatus* and *Ascaris lumbricoides* (pig strain). The saccharogenic and amyloclastic actions of these enzymes have been examined in relation to the salts present and hydrogen ion concentration.

2. Potassium iodide was found to assist the amyloclastic action of *S. edentatus* amylase to the greatest extent. In the case of *A. lumbricoides* amylase, $NaHCO_3$ was most effective. Sodium chloride did not activate the enzymes to any extent but in both parasites, Na_2HPO_4 was fairly effective.

3. Amyloclastic and saccharogenic activity was greatest at *p*H 9·4 (*A. lumbricoides*) and *p*H 8·0 (*S. edentatus*). In the latter case, saccharogenic action on a starch substrate gave rise to dextrose.

4. Sucrose action was found to be lacking or very slight in both parasites.

5. It is concluded that the amylolytic enzyme of *A. lumbricoides* is distinct from that of *S. edentatus* and that of its host also. The situations of the parasites in their hosts are such that the reaction of host juices ingested would give optimum conditions for the action of the parasites' carbohydrate splitting enzymes.

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per 100·2 grms. of mucosa. The probable sources of this zinc have been discussed.

5. Calculation has shown that *S. edentatus* must ingest from 3·9 to 21·2 grms. of horse mucosa (or 53 to 282 times the average worm weight) to obtain the amounts of zinc found. In the case of *S. vulgaris*, 0·7 to 3·4 grms. of mucosa (or 62 to 284 times the average worm weight) are necessary.

6. The fact that zinc appears to be accumulated in the parasites and is probably associated with sulphur has led to the suggestion that the zinc may be of some physiological importance, possibly acting as a sulphur acceptor in sulphur metabolism.

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The author is greatly indebted to Professor R. T. Leiper for criticism and advice on the interpretation of results. Thanks are also due to Professor D. Keilin and Dr. C. T. Calam.

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Variation in Dilution-Counts of Helminth Eggs.

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Introduction.

Helminthic egg-counts are widely used to gauge the intensity of worm infestations, especially in estimating the efficacy of anthelmintic treatment or preventive measures. In very light infestations it is customary to use one of the concentration methods, which aim at recovering (by sedimentation or flotation or both) all the eggs from a given weight or volume of faeces. These methods become tedious, however, in heavy infestations, where Stoll's dilution method, or some modification of it, is more expeditious. Here, the eggs from a given weight or volume of faeces are suspended as uniformly as possible in a considerable volume of liquid, which ideally has a specific gravity close to that of the eggs, and eggs are counted from an aliquot portion of the whole volume, removed with a calibrated pipette such as the McDonald pipette or transferred to a calibrated counting slide such as the McMaster slide.

Within recent months have appeared in various parts of the world numerous papers on the new anthelmintic, Phenothiazine. In the aggregate these papers testify to an enormous expenditure of time, labour, and money, directed towards finding the minimum effective dose against different parasites in different hosts, towards comparing different forms of the drug, and towards selecting the best of many modes of administration of the drug or of preparing the host to receive it. In one respect this phenothiazine crusade is exactly like the many crusades waged in the past on behalf of the fashionable anthelmintic of the day: the results of the numerous experiments (usually in the form of egg- or worm-counts) are hastily presented in an undigested form. In the majority of cases

no attempt whatever is made to assess the significance of the treatment differences obtained, and—lest someone else should do it for them—the authors do not reveal their actual data but refer cryptically to means and percentage reductions. Repeatedly the reader wonders whether it makes any significant difference, to give the dose all at once or in three parts, to give the drug as a drench or in capsules, to give it plain or mixed with this or that; almost invariably there is no definite answer. Heavy doses are obviously effective, but when a light dose has been tested on a few animals, has the worm-burden really been reduced a little, or is the reduction such as might reasonably be ascribed to mere random variation? The fact is that random variation is usually not discussed; the authors must know that it occurs but they avert their attention from it almost as if it were a thing to be ashamed of. Possibly this explains their reluctance to publish actual counts. Successive counts from the same material jump about in an alarming way, especially when the mean is low: Tables I and IV in the present paper illustrate this point. Whereas means of several counts are naturally steadier and look more convincing in cold print. Nevertheless, we plead for the publication of actual counts, or at the least for a satisfactory measurement of the variation involved. Only in this way is it possible to assess the significance of treatment differences.

In addition to the variation among successive dilution-counts from the same suspension of eggs, there are other factors which give rise to variation. Such are the up-and-down variation from day to day (as distinct from a general trend due, say, to a gradually disappearing infestation), variation among samples of faeces collected at different times of day, or among different portions of the same motion of faeces. Any one of these factors may or may not be significantly great in comparison with the first: it is not a matter of opinion, but a question capable of a definite answer at a definite level of precision.

The variation among successive counts from the same suspension of eggs is fundamental to all experiments using dilution-counts, and the first part of this paper is concerned with the distribution of such counts. Briefly, two questions have been asked: (i) What is the form taken by such a distribution? (ii) It is likely that the standard deviation (which is the statistic measuring the degree of variation) will vary according to the size of the mean: What is the relationship between these two statistics?

The second part of the paper describes an experiment designed to investigate some of the many other factors contributing to variation, and also to compare two methods of counting.

We have been jointly responsible for planning the work. The egg-counts for the first part were made by Mr. G. C. Martin, who shared with the junior author the counting for the second part. The calculations for the first part were mostly carried out by the junior, and for the second by the senior author, who is also responsible for checking and presentation. We are indebted to the Department of Epidemiology and Vital Statistics at the London School of Hygiene and Tropical Medicine for the invaluable loan of a calculating machine.

The Distribution of Dilution-Counts.

Technique.

A single sheep having a pure-line infestation of *Haemonchus contortus* was used as the source of the eggs counted. A given weight of faeces was thoroughly mixed with water with a mortar and pestle, and the mixture was passed through a sieve of 1/90 inch mesh to remove the coarser particles. The latter were washed repeatedly in the sieve and the whole of the suspension passing through was allowed to settle overnight. The supernatant liquid, with most of the soluble colouring matter, was then decanted off, the sediment was shaken up with a given volume of water, and 200 ml. of the resulting suspension were poured into a 250 ml. measuring cylinder. The weight of faeces taken, varying from 5 to 20 gm. and the volume of water used to dilute the sediment were so selected as to give a series of different egg-concentrations yielding mean counts from about 1 to 90 eggs per 0·15 ml. It should be emphasized that we were not concerned with the numbers of eggs per gramme of faeces, but merely with the distribution of counts in samples of 0·15 ml. withdrawn from 200 ml. of suspension.

Before withdrawing each 0·15 ml. of suspension with a McDonald pipette the suspension was agitated by 40 complete strokes with a plunger the perforated disc of which was slightly smaller than the internal diameter of the cylinder; additional strokes were continued while the pipette was being filled. During agitation the disc was not lifted above the surface of the suspension since by so doing air bubbles were trapped in it and thence into the pipette. This method of mixing has been shown statistically to be as good as pouring the suspension back and forth

between two vessels. The 0·15 ml. of liquid was delivered on to a 3×2 in. slide, a large coverglass was superimposed, and all the eggs were counted. There were 11 series of 25 counts each, each series being made from a single volume of 200 ml. of a suspension having an egg-concentration different from the rest. On the completion of each series about 4 ml. had been removed from the original 200 ml. and it is probably safe to consider the 25 counts as independent random samples withdrawn from 200 ml.

THE DISTRIBUTION OF 275 COUNTS.

The 11 series of 25 counts, together with the mean, variance, and standard deviation for each series, are set out in Table I. It will be seen that the variation within any one series is considerable; in fact the standard deviation expressed as a percentage of the mean varies from about 10% for the higher means to about 80% for the lower. On the other hand the standard deviations themselves increase with increasing values of the mean. The 25 counts of a single series are too few to give much information about the form of the distribution, and the total of 275 counts cannot be used as it stands since each series is centred about a different mean with a different standard deviation. These two sources of heterogeneity can be dealt with by first subtracting from each count the value of its appropriate serial mean, thus reducing each mean to zero, and by secondly dividing each of the resulting deviations by its appropriate standard deviation. This yields a single distribution of 275 values which, if it were "Normal" in form, would have zero mean and unit standard deviation. The actual values for this compounded distribution were: mean, +0·0055; standard deviation, 0·9894. These do not differ significantly from the theoretical values. By choosing a suitable interval, in this case 0·5 units, these "normal deviates" can now be sorted into groups to form the histogram of Figure 1, the frequencies of which are set out in Table II.

Strictly speaking the present data cannot be normal in distribution since the variate is discontinuous (the unit is a single egg), and only continuous variates can be distributed normally. The most likely form of the distribution is that of the Poisson series, which has been shown to apply to such data as blood-cell counts in a haemacytometer. One feature of the Poisson distribution is that it is completely specified by the mean; the normal distribution requires the standard deviation as an additional specification, whilst in the Poisson distribution the standard

TABLE I.

11 series of 25 counts each.

Series :	1	2	3	4	5	6	7	8	9	10	11
	1	2	5	6	9	11	17	35	40	66	86
	0	2	2	6	7	10	15	30	32	69	82
	1	0	8	3	8	16	21	27	36	71	93
	2	1	2	6	4	7	15	31	31	70	93
	1	3	4	2	6	7	9	23	35	67	92
	2	2	4	7	10	12	16	25	42	86	83
	2	3	1	7	7	12	10	28	47	56	79
	2	2	3	4	9	16	11	20	38	57	87
	0	1	5	8	10	16	17	20	39	69	78
	1	4	1	3	7	7	15	27	40	64	71
	0	4	4	6	5	9	9	28	38	64	83
	0	3	3	7	9	11	10	24	48	63	94
	1	2	5	8	9	14	20	15	39	84	65
	0	2	7	6	7	12	17	27	44	77	82
	2	2	5	4	10	10	13	23	45	62	92
	0	3	5	2	5	12	15	28	31	55	77
	3	0	4	8	12	5	15	24	38	66	73
	0	1	4	2	6	9	16	36	34	57	98
	1	1	7	4	5	10	18	25	39	68	106
	1	3	5	3	14	11	18	25	34	50	85
	1	0	3	3	6	10	13	36	41	64	96
	1	1	5	4	4	19	18	33	39	93	81
	1	3	6	8	14	7	10	33	40	74	91
	2	2	2	3	10	14	14	19	27	60	78
	1	1	7	3	8	6	18	23	47	65	108
Mean :	1·04	1·92	4·28	4·92	8·04	10·92	14·80	26·60	38·56	67·08	86·12
Variance	0·707	1·327	3·543	4·493	7·623	12·41	11·76	29·42	28·34	99·41	94·03
St. Dev. :	0·841	1·152	1·882	2·120	2·761	3·523	3·428	5·424	5·324	9·970	9·697
V per cent. :	81	60	44	43	34	32	23	20	14	15	11

deviation is numerically equal to the square root of the mean. Another feature of the Poisson series is that the appropriate frequency-histogram is skew, with the peak of the distribution lying below the mean, so that values below the mean are more frequent than those above it. This feature is marked when the mean is low (say, below 15) but as the mean increases the skewness becomes less apparent and the distribution approaches more and more closely to the normal in form. With a high mean the Poisson distribution might be considered for practical purposes as a normal one with the variance numerically equal to the mean.

We shall now attempt to show that the 275 counts are in approximate agreement with the corresponding normal curve, and that the variance is not far removed in value from the mean. Returning now to Figure 1, upon the histogram has been superimposed the corresponding normal curve, by using a table of the normal ordinates, and it may be judged by eye that the distribution is roughly normal in form. This point has been tested by comparing the observed frequencies in each interval with the frequencies expected on normal theory, using the χ^2 test. If x is the observed and f the expected frequency in each interval, then :

$$\chi^2 = S\left\{(x-f)^2 / f\right\},$$

where S denotes summation of the 8 values (the extreme frequencies are too small for the χ^2 test and have therefore been combined with their neighbours, making 8 values instead of 10). The total $\chi^2 = 2{\cdot}3762$, which for 7 degrees of freedom corresponds to a probability of about 0·9 ; in other words, as great a discrepancy between hypothesis and observation would arise merely by chance in about 90% of similar trials, so that we are justified in concluding that the distribution of 275 counts is close to the normal in form.

RELATIONSHIP BETWEEN MEAN AND STANDARD DEVIATION.

Inspection of Table I shows that, very roughly, the standard deviation approximates to the square root of the mean, as required by the Poisson distribution. It would be possible to compare the logarithms of means and standard deviations respectively in order to investigate the relationship between them, and this was in fact our original plan. But a simpler way would be to compare the variance (the square of the standard deviation) with the mean for each series, since on Poisson theory these should be the same, making allowance for random errors. This has been done in Figure 2 where the small circles are the plotted points of mean and

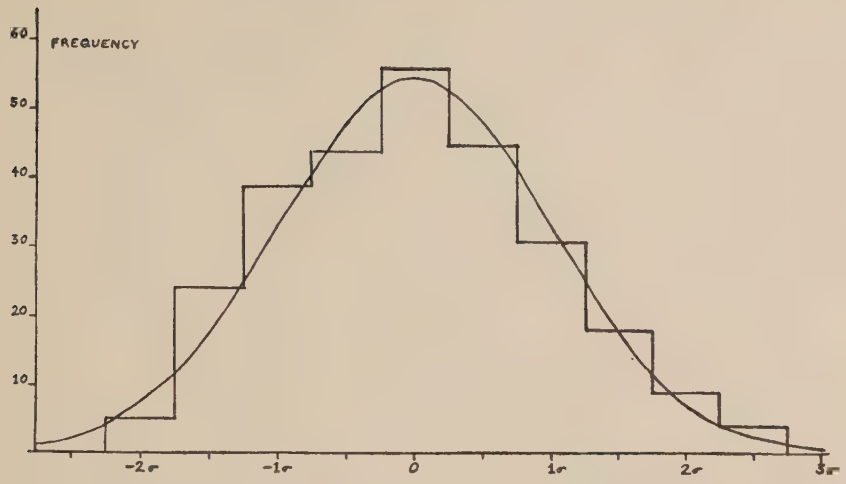

Fig. 1. Distribution of 275 counts expressed as Normal deviates; corresponding Normal curve superimposed.

TABLE II.

Frequency of " Normal Deviates " compared with Normal expectation.

Mean of Group	Frequency		χ^2
	Observed	Expected	
—2·0 to —1·5	29	28·386	0·0133
—1·0	39	33·275	0·9850
—0·5	44	48·414	0·4024
0	56	54·849	0·0242
0·5	45	48·414	0·2407
1·0	31	33·275	0·1555
1·5	18	17·806	0·0021
2·0 to 2·5	13	10·581	0·5530
Totals :	275	275·000	2·3762

variance for each of the 11 series. The points are very scattered, but it is possible to find the best-fitting straight line from the regression of variance on mean. Putting x=mean, y=variance, $\bar{x}$=mean of all the means, and $\bar{y}$=mean of all the variances, we may take as the coefficient of regression, along with its standard error :

$$b = \frac{S(xy)-n\bar{x}\bar{y}}{S(x^2)-n\bar{x}^2} \pm \frac{S(y-Y)^2}{(n-2).\ S(x-\bar{x})^2},$$

where n=the number of pairs of values and Y=the position on the regression line in terms of y of any point corresponding to a given value of x. The equation of the line is then :

$$Y=a+b\ (x-\bar{x}),$$

where the constant a is taken as $\bar{y}$. In the present case :

$$b=1{\cdot}2192\pm0{\cdot}1016, \text{ and}$$
$$Y=26{\cdot}6406+1{\cdot}2192\ (x-24{\cdot}025).$$

This is the solid line of the Figure on which the point corresponding to $\bar{x}$, $\bar{y}$ is marked by a cross. This line is subject to errors in both constants, a and b. Errors in a permit the line to shift parallel to itself, and errors in b permit it to swing about the means ; in both cases varying amounts of error have varying probabilities. Allowing for both sources of error, the variance of the line is given by :

$$V(Y)=V(a)+(x-\bar{x})^2.\ \ V(b),$$

where the variances of a and b are respectively :

$$V(a)=S(y-Y)^2\ /\ n(n-2)$$
$$V(b)=S(y-Y)^2\ /\ (n-2).\ \ S(x-\bar{x})^2,$$

and the square root of the variance for the line will be its standard error. With large samples one takes $\pm$ twice the standard error as the limits beyond which the line will lie in less than 5% of cases ; with small samples it is better to take $\pm$ t times the standard error. Thus, the 5% limits for our line will be :

$$\pm\ 2{\cdot}262\sqrt{7{\cdot}74037+(x-24{\cdot}025)^2\ 0{\cdot}010325},$$

from which the hyperbolic curves shown as broken lines have been drawn. The expected line, on Poisson theory, for mean=variance, is shown as a dotted line which just grazes the lower error-limit. Thus, we may conclude that our data are just barely consistent with the Poisson relationship.

We are indebted to Prof. R. A. Fisher, F.R.S., for pointing out that, in the case of sampling data like ours, the Poisson distribution is the ideal limit, agreement with which would indicate a satisfactory sampling

technique. Significant departure from the Poisson distribution would render the technique suspect. It may be of interest to notice that the line appears to be displaced from the Poisson line largely owing to (i) the series with the highest mean but one, which has an abnormally large variance ; (ii) most of the series with low means, the variances of which are slightly below expectation.

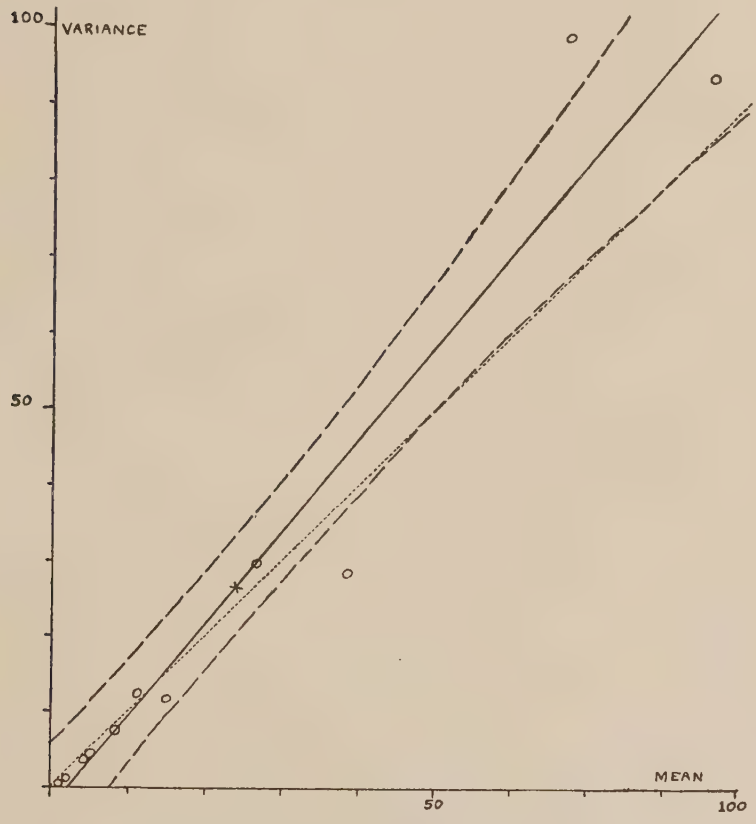

Fig. 2. Regression of Variance on Mean for 11 series of 25 counts each.

The series with low means are crowded together in a way which obscures their effect on the line. When the experiment was planned it was borne in mind that low counts are commonest in this type of sampling, and it was considered that the whole range of counts would be better examined

on a logarithmic scale. In Figure 3, x=log (mean), and y=log (standard deviation). The regression equation for the fitted solid line is :

$$Y=0{\cdot}50507+0{\cdot}55845\ (x-1{\cdot}04638),$$

and the 5% error limits, shown again as broken lines, are :

$$\pm\ 2{\cdot}262\sqrt{0{\cdot}0001511+(x-1{\cdot}04638)^2\ 0{\cdot}00043644}.$$

The dotted line represents the Poisson line corresponding to $Y=\frac{1}{2}x$, i.e., standard deviation equals square root of mean.

It will be noticed that on this logarithmic plot the discrepancy is largely due to the series with low means, the standard deviations of which are below expectation : it is in the region of these series that the expected line just crosses the upper 5% limit and runs roughly parallel to it (the extremities of the error curves are practically straight lines.).

Neither in the direct nor in the logarithmic plot is the point for the means, x and y, far from the expected line. In both cases the error consists mainly in the increased angle made by the fitted line with the x axis. This angle is measured by the regression coefficient (b=tan θ), and it might be of interest to compare the observed values of b with the values expected on Poisson theory. Even complete identity would not prove agreement between the lines, since the error in $\bar{y}$ might be sufficient to prevent this, but in the present case the error in $\bar{y}$ is not large. For the direct plot of mean against variance the expected value of b is unity (θ=45°). The ratio of the difference between observed and expected b to its standard error, taken as the standard error of observed b, is :

$$t=0{\cdot}2192\ /\ 0{\cdot}1016,=2{\cdot}157.$$

For the logarithmic plot the expected value of b is 0·5, and the ratio as before is :

$$t=0{\cdot}05845/0{\cdot}02089,=2{\cdot}798.$$

The tabulated value of t for Probability=0·05 and 9 degrees of freedom is 2·262, so that, at the 5% level of significance, the angle of the line relating mean and variance does not differ significantly from the angle of the Poisson line. That relating log $\bar{x}$ and log s does differ from the Poisson line, such a discrepancy arising by mere chance only once in just under 50 cases. This is true in spite of the fact that the absolute discrepancy is obviously larger in the first case : it appears that the logarithmic test is the more sensitive, probably because the values are more evenly spaced over their range.

For means up to about 15 the observed standard deviations fall below the Poisson expectations. Therefore, in sampling by the method here

described, one would err on the safe side by assuming the variance equal to the mean for counts of this order. For much larger counts it would be wiser to calculate the variance, or possibly to take it from one of the fitted regression lines. Extrapolation beyond the range of our counts is not advisable. In general, it may be concluded that the distribution of counts of eggs, following our technique, is not far removed from the form given by the corresponding Poisson series.

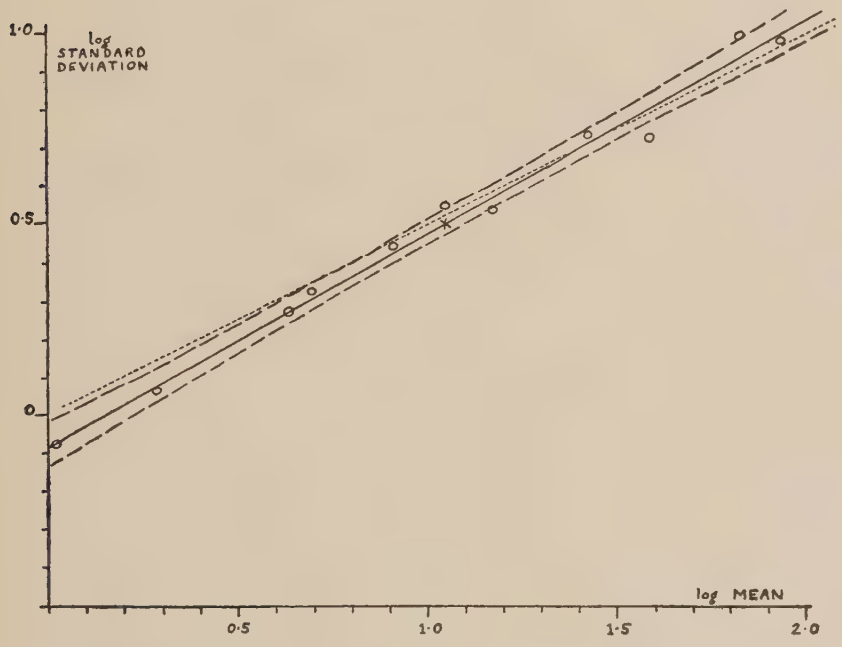

Fig. 3. Regression of log (Standard Deviation) on log (Mean) for 11 series of 25 counts each.

Other Factors contributing to Variation.

The experiment described in this second part of the paper was designed to investigate the effect upon egg-counts of day to day variation, of variation from one portion to another in the same motion of faeces, and of variation due to the use of two different counting methods. The experiment demanded the use of two sheep in order to supply sufficient faeces, so that variation among hosts was also included although we were not specially interested in this factor. In order to get through a number

of counts in one day it was necessary to employ two counters, and the experiment was therefore designed so as to keep this factor unconfounded with the others. Significant variation was not expected on this score, and was not found : it is probable that significant differences between counters would occur only with high individual counts. The experiment was quite small, involving only 192 counts spread over four successive days, but we were anxious to discover what useful information might be extracted from this type of investigation.

TECHNIQUE.

Two male lambs born in February, and carrying naturally-acquired mixed infestations, were taken at random from a flock that had been out at pasture since birth. Faeces were collected from each lamb on each of four successive days by tying on faeces-bags at about 3.0 p.m. and removing them at about 4.0 p.m. Variation from one time of day to another was not included in the experiment, and we therefore wished to exclude its effects. On the one occasion when a lamb failed to oblige, faeces were removed from the rectum. The faeces were turned out of the bags and two lots of 6 gm. were weighed out from different parts of the same motion. Each lot was mixed with water and coarsely sieved, as described in the first part of this paper, and on the following day made up to 90 ml. in a 100 ml. measuring cylinder. After thorough mixing by repeated inversion 45 ml. were poured off into a second cylinder and the remaining 45 ml. made up again to 90 ml. This suspension, containing the equivalent of 3 gm. of faeces, was sampled by the McDonald pipette (as previously described) three times by each of the two counters, giving six counts by this technique for each lot of faeces. To the second cylinder containing 45 ml. of suspension were added 45 ml. of a saturated salt solution, after which the suspension was sampled by the McMaster counting-slide three times by each counter, giving six counts by this second technique, or 12 counts in all for each of two lots, from each of two sheep, on each of four successive days.

The McMaster slide has a fixed coverslip, with a 1×1 cm. square engraved upon its under surface, suspended 1·5 mm. above the slide, so that the volume under the square is 0·15 c.c., or practically 0·15 ml. Hence both techniques involve counting eggs in the same volume of liquid. For convenience of reference we have called the two techniques " Stoll " and " McMaster " respectively, though we have adhered rigidly

neither to the Stoll method (or Taylor's modification of it) nor to the McMaster. Each method, carried out as an orthodox rite, involves a number of small and largely ceremonial acts which are probably of minor importance. The McMaster method, for example, requires that the suspension be shaken in a $\frac{1}{4}$-pint glass cream jar with 50 steel ball bearings of 6 mm. diameter. It is only right that full details of a new technique should be published, but to have investigated the effect of all these minutiae would have complicated our experiment beyond all reason. We contented ourselves with finding whether the counts were affected by using a 50%-saturated salt solution in place of water and then measuring the 0·15 c.c., and counting, with a McMaster slide. In all other respects (weight of faeces, volume of liquid, method of mixing) the techniques were made comparable. The lambs had mixed infestations, but eggs of the trichostrongyle type predominated and only these were counted.

After these counts had been made 30 ml. of the water-suspension (left over from the Stoll method) in one of the two lots from each sheep were removed, the eggs were sedimented, floated up with sugar solution by centrifuge, removed to a slide by repeatedly looping the surface, and counted. This concentration-count was intended to form a standard with which the two sets of dilution-counts could be compared.

It is well known that faeces vary from day to day in the proportion of water which they contain. Even if significant daily variation in egg-counts could be proved, this might be due wholly or partly to variation in the water-content of the faeces : the wetter the faeces, the lower the count. In order to test this possibility about 20 gm. of faeces from each sheep on each day were transferred at once to a glass-stoppered weighing jar, weighed, dried at 105°C. for 24 hours, and weighed again (constant weight was attained within 12 hours). By this means a set of counts on a dry basis was obtained, involving the use of eight correction factors (four days by two sheep). By relating the general mean of this series to the general mean of the wet-basis counts, the two series were made comparable.

STATISTICAL METHOD.

The analysis of variance for this experiment may require some explanation. In the majority of practical tests involving egg-counts one is concerned with the variation within the data for a single sheep : even where many sheep are used, the question usually arising is whether as

individuals they have responded to treatment. We used two sheep merely to supply sufficient faeces and not to investigate variation between sheep. The primary division of the data is therefore that between the two sheep, and all subsequent problems involve what happens within the data for a single sheep. As shown in Table III there are means and totals of 96 values for each sheep and one degree of freedom for the variance between them. For the day to day variation within a single sheep

TABLE III.

Scheme for Analysis of Variance.

Let X = Total, and $\bar{x}$ = Mean of 192 values for whole experiment,
" S = " " $\bar{s}$ = " " 96 " " each sheep,
" D = " " $\bar{d}$ = " " 24 " " " day (per sheep),
" L = " " $\bar{l}$ = " " 12 " " " lot (per day per sheep),
" T = " " $\bar{t}$ = " " 96 " " " technique,
" B = " " $\bar{b}$ = " " 6 " " " batch.

ANALYSIS.

Source of Variation.	Sum of Squares.	Degrees of Freedom.
1. Between sheep	$S(S\bar{s}) - X\bar{x}$	1
2. " days	$S(D\bar{d} - S(S\bar{s})$	6
3. " lots	$S(L\bar{l}) - S(D\bar{d})$	8
4. " series	$S(L\bar{l}) - X\bar{x}$	15
5. " techniques ...	$S(T\bar{t}) - X\bar{x}$	1
6. Interaction 4 and 5 ...	7. — (4.+5.)	15
7. Between batches	$S(B\bar{b}) - X\bar{x}$	31
8. Within batches (Error) ...	$S(x^2) - S(B\bar{b})$	160
9. Total	$S(x^2) - X\bar{x}$	191

there are 4 daily totals and means of 24 values for each sheep, and each sheep contributes 3 degrees of freedom making 6 in all. Similarly, interest in the random lots of faeces from each motion centres in the variation between the two lots from any one sheep on any one day : lots are therefore subsidiary to days as days are to sheep, these three sources of variation forming a hierarchy of subordinate factors rather than a set of co-ordinate factors along with their interactions. For lots there are totals and means of 12 values per day per sheep ; each pair of lots contributes one degree of freedom on each of four days for each of two sheep, or 8 degrees in all.

With the two techniques and counters the matter is otherwise : we are interested in the variation, not within single lots, but throughout the

whole experiment. The variance due to counters proved to be trivial, and this factor has been omitted from the scheme with a great consequent simplification of the analysis. For techniques there are therefore only two totals and means of 96 values each, and only one degree of freedom. It is simpler to regard this part of the analysis as if it were a separate scheme. In effect, the data form a two-way table with the two techniques (in batches of 6 counts each) in 16 series of replications. Techniques and

TABLE IV.

192 Dilution-Counts for Analysis of " Other Factors."

Day.	Counter.	Sheep I.				Sheep II.			
		Lot 1.		Lot 2.		Lot 1.		Lot 2.	
		Stoll.	McM.	Stoll.	McM.	Stoll.	McM.	Stoll.	McM.
1	A	6	4	2	3	2	5	4	7
		3	7	2	2	5	8	10	6
		2	4	2	0	1	6	4	3
	B	6	2	4	5	3	5	4	6
		3	12	1	3	3	6	6	4
		0	6	2	3	7	7	9	5
2	A	2	6	1	2	4	9	4	3
		0	3	3	1	3	2	0	4
		2	6	3	2	5	6	0	4
	B	1	2	2	3	0	2	1	5
		1	4	1	3	0	3	3	2
		0	4	1	1	3	5	9	3
3	A	3	4	2	4	13	8	2	6
		2	3	2	1	8	14	0	5
		1	1	4	3	13	8	7	4
	B	1	1	6	8	11	8	2	5
		4	2	1	3	16	16	2	5
		4	1	2	5	8	10	7	3
4	A	1	3	3	3	3	7	3	2
		2	2	1	4	4	3	3	6
		0	1	1	2	7	3	5	4
	B	1	0	5	6	2	2	3	5
		1	3	1	5	5	5	6	6
		3	4	3	1	1	4	2	5

series are therefore co-ordinate factors and there will be interaction between them. The 16 series are in fact the 16 lots, but considered in a general way so as to include also the variation due to days and sheep : the sum of squares and degrees of freedom for series will therefore be the total of those for lots, days and sheep (Table III, line 4 of the analysis). There are 32 batches of 6 counts each, contributing 31 degrees of freedom

of which 15 will be allotted to the 16 series, one to the two techniques, and 15 to the interaction between them. Finally, each of the 32 batches of 6 counts will contribute 5 degrees of freedom, or 160 in all, for determining the error variance, *i.e.*, that occurring within batches of 6. This error variance is strictly comparable with the variance discussed in the first part of this paper : it is that found among successive samples of the same suspension of eggs.

COMMENT ON RESULTS.

The actual counts are set out in Table IV. In order to save space the various sectional totals and means are not included but are readily ascertainable from the counts. For the same reason, the counts adjusted to a dry basis are not set out, but can be calculated from the percentages of dry matter given in Table V. The analyses of variance for both wet and dry counts are given in Table VI, where the dry-basis values are based on the counts that would have resulted if 3 gm. of dry faeces had been used : if x is any wet count and y the appropriate percentage of dry matter, then $100x/y$ is the dry-basis value. The ratio of the mean of all the wet counts to that of all the dry is 0·2048, and any dry-basis mean or total (or standard deviation) must be multiplied by this factor for comparison with the corresponding wet-basis value.

In Table VI the sums of squares and degrees of freedom are as arranged in Table III. The column of variances (or " mean squares ") is got by dividing each sum of squares by its available degrees of freedom. In the last column the variances for sheep, days, and lots are expressed as ratios to the error variance : unless these variances are significantly greater than the error variance one is not justified in seeking to establish differences between their means or totals. As it happens, all three are significant as judged by the value of the ratio which might occur by chance in 1% of similar trials—a stringent test (Tables of the variance ratio are given in Fisher & Yates, odd pages from 29 to 35). The technique variance is properly to be compared with the interaction variance, and is also significant at the 1% point. The same is true of the dry-basis counts, so that we may conclude at once that daily variation is not to be wholly explained by the varying dryness of faeces. Having established that these different variances are significantly larger than could be ascribed to the error variation, we are now justified in using the square

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Methods for the Recovery and Counting of Cysts of *Heterodera schachtii* from Soil.

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Introduction.

Up to the present time two methods have been described for the recovery of the cysts of *Heterodera schachtii* from soil. Carroll (1933) depended on the collection of cysts from infected roots, while Morgan (1925) floated up cysts from dry soil in one litre flasks. Powell (1939) refers to a method not yet described by which he found it possible to recover cysts from several pounds of soil at a time. The counting of cysts in soil was accomplished by modifications of the technique described by Morgan (1925). Briefly speaking it consists of floating up 50 gms. of air dried soil in water in a litre flask, rolling off the cysts from the float after drying the latter and counting the cysts in it.

The experiments to be described have for their aim, (1) the evolution of a method by which cysts could be recovered in large numbers with a minimum effort from large quantities of soil, and (2) the development of a technique for the rapid and accurate counting of the number of cysts in a larger sample of soil. With these ends in view, the present paper may be divided into three parts :

(1) Methods of handling soil in large quantities
(2) Methods of recovering cysts from the floated material
(3) Modifications in the above for counting.

The Handling of Soil in large quantities.

To test out the possibility of handling large quantities of soil at a time innumerable experiments were performed and finally five methods were evolved. These are described below.

1*st method.*—As Morgan obtained cysts by simple flotation of dry soil in flasks, the first and most obvious method of increasing the amount of soil handled at a time was to increase the size of the flotation vessel Accordingly a five gallon spirit can was used. This can was approximately

two foot deep, the upper part of it being conical, the angle of slope being approximately 40° with the vertical. Previous observations with a variety of flasks showed that at this angle cysts were not held up by the slope and prevented from floating. To the neck of this can a metal collar, with a spout of the non-drip variety, was soldered (Fig. 1). Dry soil was placed in the can, shaken for five minutes with a small volume of water to break up and wet the soil particles thoroughly and the can was then filled to the brim with water. When a period of time long enough for all the cysts to float up had elapsed, a glass tube connected to the water supply was submerged below the level of the water in the can and a slow stream of water passed in. The float then overflowed *via* the spout and was collected in a 50 mesh sieve. Experiment showed that 15 minutes sufficed for all the cysts to float to the surface while the optimum quantity of soil which could be handled per float was found to be 10 lbs. The results of a series of experiments using this quantity of soil and allowing 15 minutes for the float to rise are given in Table 1. Five lots of soil, each ten lbs., were floated in the can and the float removed after 15 minutes; a further 15 minutes were allowed to elapse and any further float removed. Half of the supernatant was then removed and the soil again shaken up for five minutes with the liquid left in the can. The supernatant fluid was then replaced and the can allowed to stand, the float being again removed after 15 minutes. All floats were taken, dried, rolled and counted. Table 1 shows that the cysts floated up after 15 minutes had elapsed were negligible in number while those floated up in the second flotation represented less than 2% of the first float.

Table 1.

Tests on Flotation in the Five Gallon Can.

	1	2	3	4	5
Cysts in 1st 15 mins.	3,600	3,926	3,646	3,221	3,917
Cysts in 2nd 15 mins.	3	0	1	2	0
Cysts in 2nd flotation	41	36	47	21	62

2nd method.—One of the difficulties attendant on the flotation of dry soil in quantity lies in drying the soil. It was therefore decided to investigate the possibility of floating up cysts from wet soil. This was done in the can mentioned in the last section, soil in varying degrees of humidity being used. It was found that when the soil was very wet, the

efficiency of flotation was very low compared with that of flotation of dry soil, and that 20% moisture was the limit as far as flotation of cysts was concerned and even at this content only about 40% of the cysts present were recovered. With a moisture content of 5–10% the recovery was fairly good, the efficiency being approximately 70% of that from air dried soil.

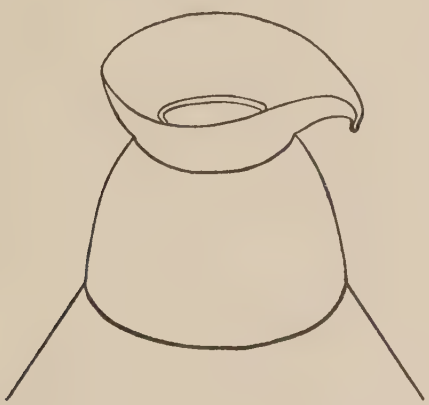

FIG. 1.—Diagram of neck of 5 gallon can.

The *3rd method* was suggested by Professor Leiper to increase the efficiency of wet floating. Wet soil was washed through a 25 mesh sieve and the washings passed through a 50 mesh. The fraction of soil collecting in the latter was then shaken up with water in a tall glass vessel. On standing the main mass of soil sank to the bottom almost immediately, but the wet cysts took longer to do so. Quick decantation of the supernatant fluid into a 50 mesh sieve resulted in the transfer of the greater part of the cysts to the latter. The shaking and decantation was repeated three times when it was considered that the majority of the cysts which had not sunk were collected. This method was very easy to apply and a large volume of soil could be handled in a very short space of time, but it was found that a very large amount of débris was recovered with the cysts.

4th method.—While the three previous methods have certain advantages over the flask technique all suffer from one serious disadvantage, viz., they are not continuous. As method 3 was found to be very efficient as far as the number of cysts recovered was concerned and moreover could be

applied to wet soil it was decided that the next step should be to see whether the process could not be made continuous and an apparatus was finally evolved to this end. The apparatus to be described works fundamentally on the same principle as that underlying method 3 except that separation of the float from the soil is accomplished before the second sieving, but the shaking of the soil with water in a tall glass vessel is dispensed with, the same effect being obtained by means of a current of water passing upwards through the vessel. A diagramatic representation of the apparatus is given in Fig. 2. A is a 25 mesh sieve supported by a large funnel B. C is an inverted winchester bottle, the bottom of which has been removed. The neck of the winchester is fitted with a one hole cork and tube D connected with the water supply. In the lower part of C is placed a layer of stones to render the upward flow of water through it even. The overflow from C is collected by the large funnel shute E and led to the 50 mesh sieve F. The nozzle of the funnel B is adjusted so that it is about 2 ins. below the level of the rim of C.

The method of operation is as follows ; the water supply at D is turned on and C thus fills up. When full, the water is adjusted so that a steady overflow is maintained from C. Soil is then placed in A and washed through into C by means of a jet of water. Provided the current of water up through the latter is adjusted correctly, the soil, being heavy, will sink to the bottom, while the cysts, being lighter, will be carried up in the stream and will overflow and be caught in F. More soil can be added to A until C is nearly full, the size of the latter being the only limitation as far as quantity is concerned. At the end of the experiment the current of water upwards was maintained until the overflow was clear. By this time it was considered that all but a negligible number of cysts had overflowed and been collected. Care was found to be necessary in adjusting the flow of water up through D otherwise large quantities of soil were washed over into F. If this flow was correctly adjusted the debris recovered with the cysts was surprisingly small in quantity and compared very favourably with that obtained by dry flotation.

The *5th method* is in reality a simplification of method 4, the layout of the apparatus being the same as previously except that the upward current of water was dispensed with, since it was hoped that the swirl induced by the jet of water and soil from A would carry the cysts up.

This method was found to be far simpler to operate than was the previous one and the material accompanying the cysts in F was even less in quantity than in method 4.

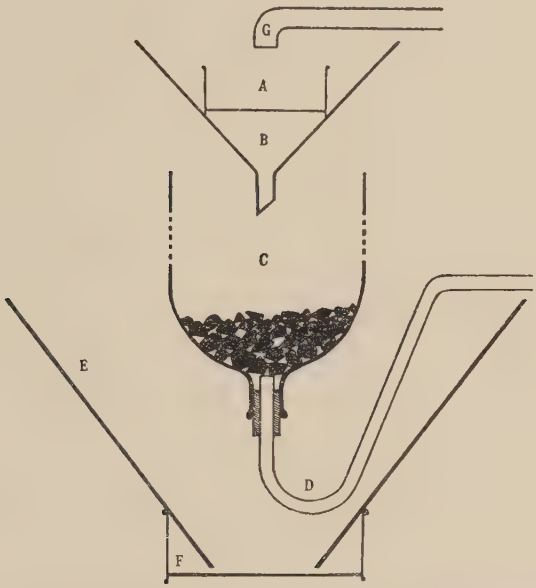

FIG. 2.—Diagram of experimental apparatus for cyst collection from wet soil.

In view of the fact that all five methods described gave quite good yields, it was decided that the only method of choosing between them was to perform a series of experiments on equal quantities of soil using each of the five methods in turn and comparing the yields from each. A binful of soil was accordingly taken and was coned and quartered four times to ensure uniformity. Three pound samples of this were then taken in five batches of six. One batch was air-dried and floated in the can, the rest were treated by the four other techniques. The water content of the soil was approximately 10%. It was not considered advisable to

use soil wetter than this in view of the very low yields obtained from very wet soil. The yields obtained from each technique are set out in Table 2.

TABLE 2.
Comparative Test on the Five Methods mentioned.

Method No.	1	2	3	4	5	6	Mean
1	1,201	1,198	1,239	1,304	1,317	1,271	1,255
2	781	762	807	790	801	782	787
3	999	1,043	1,044	1,019	1,022	986	1,019
4	1,100	1,030	1,002	986	1,060	1,126	1,051
5	962	930	946	936	959	929	943

It will be seen from the above table that the highest yield was obtained from the simple flotation of dry soil while the lowest was the result of flotation of wet soil. A statistical analysis of the figures, given later in this paper, shows that all the differences between the means are significant except between (3) and (4). These latter methods gave yields second only to (1), while (5) was the next in the list. In choosing between these methods, attention was paid to two points, yield and convenience in operation. On these grounds, (2) can be ruled out immediately in view of its very low yield and the fact that it is not continuous. In the choice between (3) and (4), preference must be given to (4) since it is a continuous process. One is now left to choose between (1), (4) and (5) and it was considered by the author that the smaller yield resulting from the least efficient of these was more than made up for by the fact that they were continuous. In choosing between (4) and (5), attention must be paid to the fact that (4) requires far more attention in operation than does (5), the latter being very simple to operate by even an unskilled person. It was accordingly decided that this was the most promising and a large apparatus was devised for the collection of cysts from large quantities of soil.

The layout of the apparatus may be seen on referring to the Plate (B) & (C). It consists of an old milk churn to which the following modifications have been made ; around the neck of the churn was soldered a metal channel furnished with a spout capable of completely draining the latter. To the rim of the churn was fixed a detachable framework supporting a large metal funnel with its nozzle about four inches below the level of the rim. A baffle plate was supported about two inches below the nozzle of

the funnel by means of four pieces of wire resting on the rim, to break up the jet of water and soil entering the can through the funnel. It was found that the use of this baffle plate resulted in a float that was free from silt and sand, which invariably accompanied in large quantities that resulting from not using the baffle plate. The most convenient method of operation was found to be as follows ; The churn was first filled with water and a large 50 mesh sieve placed under the spout. Soil was placed in a 20 mesh sieve supported on legs in the funnel. A powerful jet of water was played on it, the soil particles being broken up and washed into

Table 3.

Differential counts on cysts recovered by dry flotation and continuous flooding.

		Full	Half Full	Nearly Empty	Empty	Empty Shells	Percentage Full
1 ...	Dry	92	16	1	4	1	80·7
	Wet	90	12	8	8	2	75·0
2 ...	Dry	82	22	12	20	6	57·9
	Wet	84	20	16	31	4	54·0
3 ...	Dry	101	12	3	2	0	81·3
	Wet	94	13	4	7	1	80·4
4 ...	Dry	97	4	0	2	0	94·1
	Wet	100	7	6	4	2	84·0
5 ...	Dry	86	16	3	6	1	76·8
	Wet	88	21	2	9	1	72·7
6 ...	Dry	89	12	6	5	2	78·1
	Wet	88	13	7	8	3	73·9

the churn. The cysts and other light debris overflowed into the channel and were collected in the 50 mesh sieve, the soil, being heavier, sinking to the bottom of the can. It was found in practice that large quantities of soil could be handled by means of this apparatus very rapidly, one cwt. of soil being washed through in about two hours, and resulting in the collection of approximately 50,000–60,000 cysts.

Reference to Table 2 will show that although the yield from the method described using wet soil is comparatively high, it is still lower than that obtained by dry flotation. It was considered possible the method might

select some type of cyst rather than another ; if that were the case it was felt that the empty cysts would be the ones which the method was most likely to recover. Experiments were therefore performed to test out this possibility. Six different samples of wet soil of approximately 10% water content were taken and portions of each after thorough mixing were dried, and floated, the cysts obtained from the floats being dissected and classified into full, half full, nearly empty and empty. Those containing only empty shells were classed separately. The numbers in each class were noted. The remainder of the samples were subjected to the continuous flooding process and the cysts recovered classified and counted as before. The results are set out in Table 3. It will be seen that although the percentage of full cysts is greater from dry flotation, yet the difference between it and the percentage resulting from continuous flooding is very small. It was therefore decided unless the drying of the soil could be accomplished very easily indeed, that the labour involved was not worth the very small advantage resulting from it.

Recovery of Cysts from the floated Material.

One of the biggest difficulties experienced in the collection of cysts on a large scale was the recovery of cysts from the floated material in a clean condition. The very large quantity of float resulting from the flotation of a hundredweight of soil resulted a very great deal of very tedious work to dry and roll. It was therefore decided to investigate the possibility of recovering cysts by some easier and less tedious method.

The possibility of separating off the cysts from the dried débris was first investigated and a large number of experiments using a variety of types of apparatus were performed. In no case, however, was a satisfactory separation attained. Similar failures with other methods of separation from the dried débris eventually led the author to abandon work on the dried float, and to concentrate on finding a method of handling the material in a soaked condition. It is a well known fact that the cysts will float in a dry condition, but that on thoroughly wetting they will sink. If, therefore, the floated material is allowed to stand for several days with occasional stirring the greater part of it will sink, leaving only a few cysts still floating. The process of soaking and sinking can be hastened by the use of a filter pump, but in all cases a few cysts are left on the surface.

These cysts were examined and it was found that they were about 98% empty so that from the point of view of cyst collection they could be ignored.

The problem now remained how the cysts could be collected from the soaked sunken material ? It was found possible to eliminate a great deal of the débris by the following simple method : A convenient quantity of the material is agitated with water in a petri dish when the cysts are found to concentrate into a number of compact masses. The greater part of the débris can then be decanted off with the loss of only a very few cysts. If the process is repeated a number of times a very considerable increase in the concentration of the cysts can be attained.

For the final separation of the cysts from the sunken débris best results were obtained by differential flotation. It was found convenient to accomplish this in two stages :—first a heavy solution was used which brought up nearly all the cysts together with a small quantity of débris, to be followed by the transfer of the floated cysts to water in which they sank, and subsequent flotation from a lighter solution, to eliminate the last traces of débris. The technique of flotation was worked out using sugar solution, which did not appear to have any deleterious effect on the cysts provided they were not allowed to remain in it for long and that the sugar was well washed out afterwards. Sugar solutions used are best obtained by dilution from a standard sugar solution as used for the flotation of eggs from faeces. Such a solution is obtained by dissolving 16 oz. of domestic sugar in 12 oz. of water. The solution for the first flotation may be prepared from this by diluting 70 ccs. of this to 100 ccs. with water. It has an S.G. of 1·21. The more dilute solution for the second flotation is prepared by diluting 55 ccs. of the solution to 100 ccs. with water and has an S.G. of 1·165. If preferred the two solutions may be made up directly by dissolving 23 oz. of sugar in 27 oz. of water for the stronger solution and 37 oz. of sugar in 63 oz. of water for the more dilute solution.

It is important when recovering cysts from the float by this technique that certain conditions be observed. In the first place, the cysts should only be left in the sugar for the minimum period possible ; the quantity of material in the centrifuge tube should be comparatively small compared with the amount of fluid present, otherwise a considerable loss may result from trapping, a depth of one inch of centrifuged material is a

suitable quantity for a 15 cc. centrifuge tube ; the sugar should be washed out of the cysts very thoroughly after flotation otherwise trouble will be experienced with fungal growth on the cysts. A convenient method of washing the cysts is to place them in a long tube of about one inch diameter, the bottom of which is closed with bolting silk ; a gentle stream of water may then be played on the cysts for fifteen to twenty minutes.

As stated above this technique of flotation was worked out with sugar solution but there is no reason why some other solution of similar specific gravity should not be used. Experiments have been conducted using saturated salt solution for the first solution and 22% salt (by weight) for the second solution. The cysts obtained from this solution were also normal but far greater care was necessary as these solutions harmed the cysts in a far shorter time than did sugar solution. Saturated magnesium sulphate solution may also be used for the first solution, the strength of the second solution being 30% $MgSO_4$ $7H_2O$ by weight. This solution did not, however, appear to be so efficient as either of the other two.

It would be interesting at this stage to record the results of a series of experiments on the efficiency of sugar flotation. Two lots of ten lbs. of soil were floated up in the can and the floats recovered ; one was dried and rolled and the cysts in it collected and counted. The other was soaked until nearly all the cysts and débris was sunk. The sunken material was then treated according to the technique already outlined. The cysts recovered and the cysts lost at all stages were counted and the results are given below.

Results.

	1	2	3	4	5	6
(*a*) Cysts lost in decantation	387	429	475	321	538	467
(*b*) Cysts in residue of first flotation ...	257	307	198	206	275	384
(*c*) Cysts in residue of second flotation	576	765	695	742	598	578
(*d*) Cysts in second float	2,548	2,437	2,178	2,635	1,937	2,837
(*e*) Cysts lost through not sinking ...	419	298	357	327	442	385

Cysts rolled from the ten pound float ... 3,621.

Examination of the cysts counted revealed the fact that nearly all the cysts in (*b*) and (*e*) were empty while the percentage of cysts empty in (*c*) was approximately 80% ; the technique is thus seen to reduce the number of empty cysts recovered. An examination of the cysts recovered in (*d*)

disclosed the fact that they were 87% full while those recovered from the first float by rolling were only about 54% full.

Estimation of the Number of Cysts in a Sample.

It would be interesting to consider at this stage the standards to which any quantitative technique must conform. In the first place the method must be simple to operate and capable of giving comparable results when operated by a variety of workers ; the total yield obtained must be as high as possible and thirdly individual results must show low variability.

Table 2 gives figures for the yields obtained from three pounds of soil treated six times by each of the techniques mentioned earlier on in this paper. A statistical analysis of these figures is given in Table 4.

Table 4.

(*Analysis of figures in Table* 2.)

	Variance	Standard deviation	Standard error	Coefficient of variability
(1) ...	2584·0	50·83	20·75	4·05%
(2) ...	258·2	16·07	6·56	2·04%
(3) ...	539·8	23·23	9·49	2·28%
(4) ...	3026·6	55·02	22·46	5·23%
(5) ...	207·5	14·40	5·88	1·53%

It may be seen from this that all the differences between the means are significant except between the means for (3) and (4). All three methods giving the highest counts give also the greatest variability. (5) has the lowest variability and the lowest standard error. If a series of six counts each were done on the soil by this method then 95% of the means thus obtained would fall inside the limits of 932–956, while if single counts were done the limits in 95% of the cases would be 915–973. It was consequently decided in view of the very low variability, provided a method could be found of increasing the yield without also increasing the variability, that this should be the method adopted for cyst counting.

It has already been mentioned that the highest yield was obtained by dry floating which also gave the highest variability, the opposite being the case with method (5). It was considered that in the two methods there were two factors involved. In dry floating all the cysts would be dry and except in so far as they were prevented from so doing as a result of trapping by the soil particles on standing, they would all float to the

surface ; it is reasonable to expect this loss to be small but variable in extent. This would account for the high yield and the poor consistency. In the continuous flooding method, trapping would be reduced to a negligible extent as the cysts never reach the bottom of the can with the soil mass and are therefore perfectly free to float ; on the other hand when the experiments are performed on wet soil it is highly probable that some of the cysts might be soaked and therefore sink. Comparison of the yields from wet and dry floating indicates that the proportion of cysts which did not float in wet soil was approximately 33%, and it is highly probable that the increase in efficiency of the continuous flooding method was at least in part due to the lack of trapping in this method. It was therefore decided to determine the effect of continuous flooding on dry soil and a number of experiments to this end were performed.

A sample of approximately two cwts. of wet soil of about 8% water content was taken and separated into four main fractions by sieving, the fractions being as follows :

A greater than $\frac{1}{2}$ inch.
B $\frac{1}{2}$–$\frac{1}{4}$ inch.
C $\frac{1}{4}$–$\frac{1}{10}$ inch.
D less than $\frac{1}{10}$ inch.

The sole function of this separation to fractions was to test out the method of mixing to be described. To ensure that the cyst contents of each fraction was different from that of the others 50 gm. counts were done on each.

Results of 50 gm. counts on the fractions.

	1	2	3	4	5	Average.
A	73	88	78	53	57	76
B	110	76	86	86	73	86
C	67	75	99	91	72	81
D	128	125	117	110	107	107

The fractions were then mixed as follows :—the four fractions were roughly mixed into one pile and the pile coned and quartered four times. The four piles resulting from the last quartering were not coned but were mixed in diagonal pairs and the two heaps thus formed mixed into a long line. The line was then transferred by alternate shovelsful from each end into a bin ; the soil in this bin was then disturbed as little as possible and used for future experiments.

Three pound samples were then taken, ten samples were dried and floated in the cans, ten were dried and treated by the continuous flooding process, while two other lots of ten were floated and subjected to the continuous flooding process respectively. The floats were collected, dried, rolled and the cysts counted. The results are given in Table 5. In both figures and analyses for wet flotation it will be seen that the first result was far higher than any of the others and very far outside the general range. In the calculation of the means and in the analysis, this figure has been ignored. It will be seen that by using dried soil for the continuous flooding process the yield is higher and the variability lowered, thus confirming the suggestions already mentioned.

TABLE 5.

Comparative Tests on the Use of Wet and Dry Soil.

											Mean
Dry flotation	1594	1561	1521	1612	1520	1567	1501	1609	1663	1596	1574
Wet flotation	2099	1399	1236	1438	1443	1349	1410	1320	1276	1357	1432
Dry soil flooded	1769	1757	1742	1750	1771	1762	1773	1758	1754	1761	1759
Wet soil flooded	1401	1503	1362	1549	1521	1436	1492	1338	1426	1456	1448

Analysis of figures.

	Variance	Standard deviation	Standard error	Coefficient of variability	Mean
Dry flotation	2587	50·57	15·90	3·19%	1574
Wet flotation	5142	71·71	23·90	5·28%	1432
Dry soil flooded ...	94	9·69	3·07	0·55%	1759
Wet soil flooded ...	4765	68·03	21·81	4·70%	1448

Having determined the most efficient and most consistent method of recovering cysts for flotation, two problems remained to be solved: (1) to determine the optimum size of soil sample and (2) to determine the absolute efficiency of the method on different soils. In determining the size of sample most convenient, a balance had to be struck between the maximum size of sample and the labour involved in counting the cysts recovered. The larger the sample taken, the greater the labour involved in counting the cysts and the problem therefore resolves itself to determining the minimum size of sample which will give accurate results.

The remainder of the soil was therefore dried and a series of samples of different sizes taken and subjected to continuous flooding. For each size, ten samples were taken: the results are set out in Table 6, from which it will be seen that as the size of sample is decreased below three pounds the variability increases correspondingly, the critical point being apparently reached at half a pound, when any further decrease in the size of the sample resulted in a substantial increase in the variability, the coefficient of variability for half a pound being 1·2% and that for a quarter pound being 6·16%. Using samples of this size (½ lb.) if a series of determinations were done on single samples then 95% of the results would deviate by less than ± 2·4%; if the determinations were done on three samples than the means would deviate by ± 1·4%; if ten were taken for the determination then the deviation in the means would be ± 0·7%. It was accordingly decided that the optimum size of sample should be half a pound.

TABLE 6.

The effect of different size samples on the accuracy of counting.

											Mean
1 lb.	642	644	639	648	641	646	649	650	645	643	645
½ lb.	323	320	320	320	331	326	324	328	319	321	324
¼ lb.	164	171	163	153	165	175	156	170	174	151	164

Analysis of figures.

	Variance	Standard deviation	Standard error	Coefficient of variability	Mean
1 lb. ...	13·0	3·6	1·14	0·56%	645
½ lb. ...	15·1	3·9	1·23	1·20%	324
¼ lb. ...	73·5	8·5	2·71	6·16%	164

A small apparatus was accordingly devised to handle small quantities of soil. As will be seen on referring to the Plate (A), this was constructed on the same principle as the large apparatus. It consisted of a conical vessel about twelve inches deep and six inches diameter at the base and tapering to three inches at the top. Around the top was built a large channel sloping towards one side, the lowest side of the channel being produced into a spout which discharged into a net of bolting silk. Above the neck

of the vessel was placed a large metal funnel which supported a 20 mesh sieve into which the soil sample was placed. The method of working this apparatus was similar to that adopted for the large scale apparatus, apart from certain precautions which it was found necessary to observe. When the sample had been washed through the funnel the sieve was removed and any soil particles sticking to the sides of the funnel were washed into the can by means of a sharp jet of water. The apparatus was then allowed to stand for five minutes to allow any cysts which had not reached the surface in the can to rise. They were then flooded off into the channel by means of a gentle jet of water through the funnel. Care

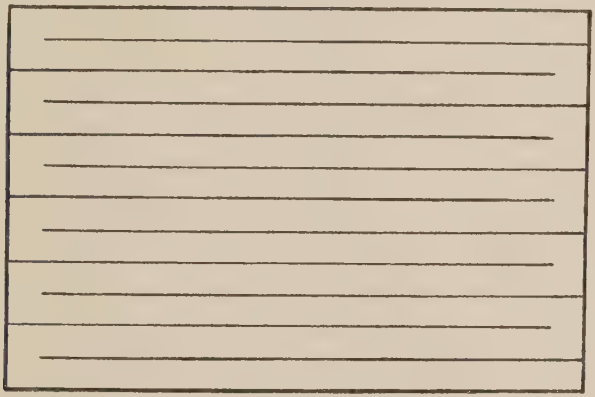

FIG. 3.—Diagram of cyst counting slide.

should be taken that the float is washed off the surface very thoroughly and that none of it is allowed to adhere to the sides of the funnel. The float collected in the bolting silk was then collected and transferred to a flask with water to allow any silt or sand washed over with the float to sink. The remaining material still floating was then recovered, dried and rolled. Experiments were performed with this apparatus to determine whether or not the accuracy of this apparatus was comparable with that obtained with the improvised apparatus used for the experiments on cysts counting and it was found that it was in every way satisfactory.

It was subsequently found that the bolting silk could be conveniently dispensed with and its place taken by a 100 mesh sieve, the interstices of

which measured 160–170μ, since measurements showed that even the smallest of cysts could not pass through this mesh.

The remaining problem to be solved was to determine the absolute efficiency of the continuous flooding method on different types of soil. Three main types were tested, an extreme sandy soil represented by pure sand, an extreme clay soil represented by pure clay, and thirdly an intermediate type. Half pound samples of each were taken and to each sample was added 500 cysts. Each sample was then wetted and dried three times and finally dried. The cysts were recovered and counted. Blank tests were also carried out on samples which had not been infected. The results are set out in Table 7. It will be seen that the efficiency of the technique was very high, being in the neighbourhood of 98% for sand and approximately 95% for pure clay, and intermediate types.

Table 7.

Tests on the Efficiency of the Apparatus with different Types of Soil.

	1	2	3	4	5	6	Mean
Pure sand before infection	0	0	0	0	0	0	0
Pure sand after infection	498	499	500	496	499	501	498·5
Recovery from pure sand							498·5
Intermediate type before infection ...	51	49	49	50	49	48	49·3
Intermediate type after infection ...	544	543	596	545	543	548	545·2
Recovery from intermediate type ...							495·9
Clay before infection	0	0	0	0	1	0	0
Clay after infection	496	496	494	499	495	496	496·0
Recovery from clay							496·0

It is considered that a refinement in counting might be fittingly described at this stage. Counting of the cysts was greatly facilitated by the use of a special counting slide. This slide can be constructed from a piece of tinplate which may easily be formed into a shallow tray measuring about 2×3 ins. The metal was cut and soldered so that the sides were sloped outwards at an angle of about 45° with the vertical. The field of the binocular microscope used was measured and lengths of wire laid along the floor of the tray parallel with its long axis at a distance apart slightly less than the field covered by the microscope. Each strip extended from one end of the tray to a distance from the other and also equal to the diameter of the field alternate strips starting from alternate ends of the tray. (Fig. 3.) A long zig-zag channel was thus formed, the

whole of which could be covered by the microscope in one long continuous to and fro movement. The presence of the raised strips of wire prevented the cysts from wandering from one part of the tray to another and thus obviated any tendency either to count a cyst twice or to miss it completely as a result of parallax, which danger is always present where the separation of a slide into regions is accomplished by means of etched or drawn lines only. The most suitable surface for this tray was found to be obtained by treating with a coat of flat white paint. With such a surface there was no appreciable glare, but the white reflected just sufficient light to model the surface of the cysts and render them easily distinguishable.

Summary and Conclusions.

(1) A method of recovering cysts from soils of not more than 10% water content is described. The method is continuous and the yield from it is only slightly lower than that from the dry flotation of soil. The yield was found to contain a slightly lower percentage of full cysts than that obtained by dry flotation but this drop was not considered sufficiently great to justify the drying of soil in large quantities. Quantities of soil in the neighbourhood of 1 cwt. can be handled continuously in a matter of two hours.

(2) A new method of recovering cysts from the float is also described, involving the soaking of the float and the recovery of the cysts from it by differential flotation.

(3) It was found possible to modify the method for cyst counting and the optimum size of sample was found to be $\frac{1}{2}$ lb. Using samples of this size, results of a known standard of accuracy may be obtained, the standards being given in the text. A new type of cyst counting slide is described.

Acknowledgments.

The author is deeply indebted to Professor R. T. Leiper, D.Sc., F.R.S., for suggesting the problem and much help and encouragement readily given the author while this work was in progress.

The author also takes this opportunity of expressing his gratitude to Mr. G. Martin for invaluable co-operation in the evolution of the techniques described and for innumerable suggestions, without which the apparatus described might never have reached its present efficiency.

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A

B

A.—Apparatus for the enumeration of cysts in small quantities of soil.
B.—Assembled apparatus for the recovery of large numbers of cysts from soil.

To face page 172.

C.—Photograph of the neck of churn showing the modifications and baffle plate.

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Observations on the Vertical Migrations of Infective Larvae of certain Bursate Nematodes.

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The migrations of infective larvae of sheep and horse Strongyles have been studied by various workers, principally in relation to their behaviour in faeces or in different types of soil. (Bruns, 1937 and Lucker, 1938). The factors affecting the extent and nature of the migrations of certain infective larvae on to grass have also received attention (Rogers, 1940). A knowledge of the behaviour of larvae both in soil and on grass is very necessary if control methods either by chemicals or by controlled pasturing are to be effective. The present paper, however, describes an attempt to study the fundamental behaviour of these larvae in the laboratory, in the hope of obtaining results of practical value or of filling up certain gaps in our knowledge of them. A simple technique was sought, whereby the migrations of the larvae could be studied in the laboratory under different conditions, and is described herein.

Technique.

After various trials and errors a simple method was devised which was fairly satisfactory and was subsequently used without variation in all the tests. A 3×1 in. glass slide was marked out in ten compartments by

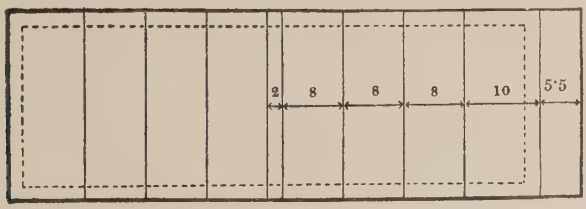

Diagram I.—Marked slide showing spacing of lines, in millimetres. The dotted line represents the vaseline border.

nine parallel transverse lines drawn with a writing diamond. The spacing of these lines is seen in diagram I. It will be seen that there is a central compartment 2 mm. in width and on each side of it are three compartments each 8 mm. wide. Beyond these are two more, each 10 mm. wide and at one end is an unpaired space of about 5·5 mm. which is used for attaching the slide. Infective larvae, obtained from horse or sheep faeces by

Leiper's method (1937), were smeared on the 2 mm. space with a small brush, or applied in a drop of water which was allowed to evaporate almost to dryness. Next, fine sand, acid treated to remove impurities and passed through a sieve of 100 meshes to an inch, was sprinkled evenly over the slide. To enable the sand to adhere temporarily to the slide, the latter was breathed upon just before being sprinkled. Permanent adhesion was later ensured by spraying with an atomiser. The amount of sand used on each slide was kept as uniform as possible and did not exceed one sand-grain in depth, nor were the grains so close together as to make subsequent counting of the larvae at all difficult. With a mounted needle the sand was next cleared away from the edge of the slide, leaving an unsanded border of about 2 mm. wide, which included the attachment portion of the slide. This border was then smeared with a thin layer of vaseline by means of a glass pipette containing vaseline and provided with a plunger instead of a rubber teat. The object of the vaseline was to prevent the larvae from migrating to the under surface of the slide, and was in the main successful, especially as minute globules of water, the result of subsequent spraying, were very effective in trapping the larvae. Breathing on the sanded slide was found inadequate for moistening purposes, so that an atomiser containing distilled water was used. Here again care had to be taken to moisten the slide evenly all over and to apply the correct amount of moisture. Too much moisture would cause drops to accumulate and run down the slide if it were held vertically ; and too little might result in evaporation before the completion of an experiment and prevent further migration. In this respect the sand served a double purpose, for in addition to being an excellent medium for the larval migrations it served to retain the moisture uniformly. To prevent evaporation of the moisture the slide was kept in a $4 \times 1\frac{1}{4}$ in. glass tube and secured there by inserting the attachment end of it into a groove cut in the cork stopper. The cork was a tight fit in the glass tube and was well smeared with vaseline before being replaced ; otherwise the air inside the tube would not remain saturated and evaporation would occur. At the end of an experiment the extent and direction of larval migration was estimated by counting the larvae in each of the nine compartments on the slide. In order to prevent further migration during the counting, which usually occupied 20 to 30 minutes, the sand grains were cleared from the lines on the slide for a width of about 1 mm. with a mounted needle. The moisture on these cleared lines quickly evaporated thus

forming a barrier between each compartment and isolating the larvae in them. The sand was then allowed to dry in order to immobilise the larvae for counting.

In the diagrams herewith, which illustrate the results of counting larvae after an experiment, each line represents a marked slide, and the dots on the line represent the transverse lines which limit the compartments. Opposite each compartment is the *percentage* number of larvae found in it and directly under the line is the total number of larvae on the slide. (In the case of horizontal slides the total number is at the left-hand side). In order more readily to see the trend of the migration, the figures in the upper three, the middle three and the lower three compartments have been added up in each case and the totals are indicated by a bracket. During the time lapse between the setting up of a slide and the attainment of the temperature required, a certain amount of migration would take place from the middle compartment to the adjoining ones, especially in the case of temperatures below that of the laboratory, and hence the lumping together of the totals of the middle three compartments in particular is desirable.

Results of Experiments.

Vertical migrations of Trichonema larvae at different temperatures.— In diagram II are seen the results of an experiment to test the influence of different temperatures on the vertical migration of infective larvae of

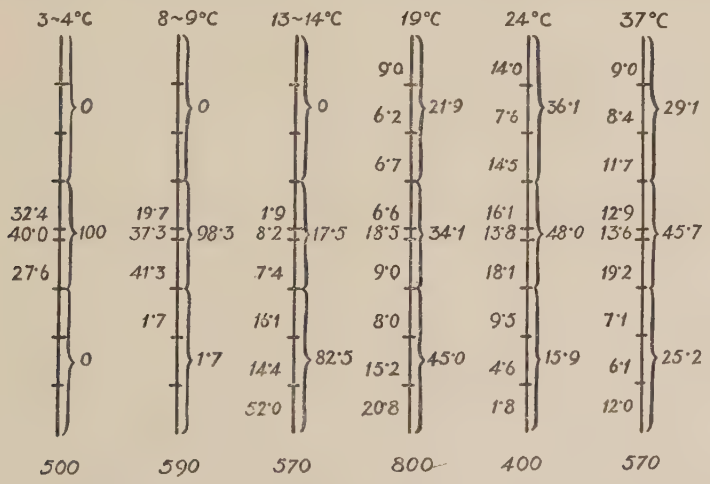

Diagram II.—Vertical migration of infective larvae of *Trichonema* at different temperatures, for 3 hours in darkness.

Trichonema. The larval culture used was a mixture of several species of *Trichonema* in which were a minute proportion of *Strongylus* larvae. The larvae were fresh and vigorous, having been cultured only five days previously. They were collected from the culture vessels in the morning and necessarily were exposed to light whilst the slides were being set up, but during the 3 hours of the experiment they were in darkness. The series shows that below 10°C. the larvae do not migrate much, a not unexpected result in view of the fact that at such a low temperature their movements are greatly slowed down. At 13–14°C. the larvae showed a very marked

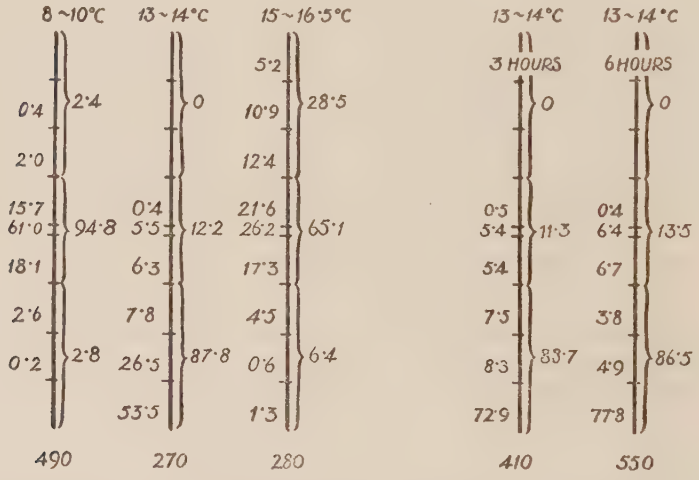

Diagram III.—Vertical migration of infective larvae of *Trichonema* at different temperatures, in daylight for 4 hours. Illustrating limiting temperatures for positive geotropism.

Diagram IV.—Vertical migration of infective larvae of *Trichonema* for 3 and 6 hours respectively at 13–14°C.

positive geotropism. At 19, 24 and 37°C., the larvae migrated both upwards and downwards ; but the downward tendency at 19°C and the slight upward tendency at 24°C. and 37°C. are not characteristic, as repetitions of this experiment have shown. The positive geotropism at 13–14°C. is extremely interesting as it invariably occurs about this temperature and is usually strongly marked. It was thought when first observed that it might have been caused artificially by the influence of gravity on larvae which are not so vigorous as at higher temperatures ;

in other words that the sluggish movements of the larvae were simply causing them to slip down between the sand grains. It was found, however, that geotropism at this temperature also occurred on slides which were not in the vertical position but merely tilted slightly at one end. Even if the slide was sloping only 1 in 8, the geotropism of the larvae was quite evident. Furthermore, actual observation under the microscope of the movement of larvae on a slide so tilted and placed in a Petri dish containing ice to give the required temperature, showed that the larvae orientated themselves with their head end towards the lower end of the slide and then proceeded down the slope. The minimum angle at which this geotropism takes effect has not been ascertained, but observations have shown that it occurs on slopes considerably less than 1 in 8. The limiting temperatures at which it occurs are not certain but they appear to lie between 10 and 15°C. as is indicated by the results shown in diagram III. In this series the slides were kept at the temperatures for four hours and were exposed to light.

The duration of the tests was in each case usually about three hours, as it was found by preliminary trials that this time was adequate for the purposes of the experiments. In diagram IV are seen the results of two identical tests for 3 and 6 hours respectively at 13–14°C. The difference between the amounts of migration in each case is insignificant.

A comparison of the effects of light and darkness on the vertical migrations of *Trichonema* larvae at different temperatures gave somewhat inconclusive results. In diagram V three of the slides were exposed to light from 12 noon to 3 p.m., and the other three were in darkness for the same period. Except for a better dispersal of larvae at 19–21°C. in light and a better downward migration in darkness there is not much difference between the two series. This better dispersal at 19–21°C. is probably due to the fact that light has a stimulating effect on the larvae. It has often been noticed that larvae left in a watch glass of water overnight are very sluggish or motionless in the morning ; but on exposure to light they quickly become active.

Vertical migrations of Haemonchus contortus larvae at different temperatures.—Many observations were also made on the vertical migration of infective larvae of *H. contortus*. Diagram VI illustrates a fairly typical result. The poor migration at 11°C. contrasts with that of *Trichonema* larvae which at this temperature would exhibit a positive

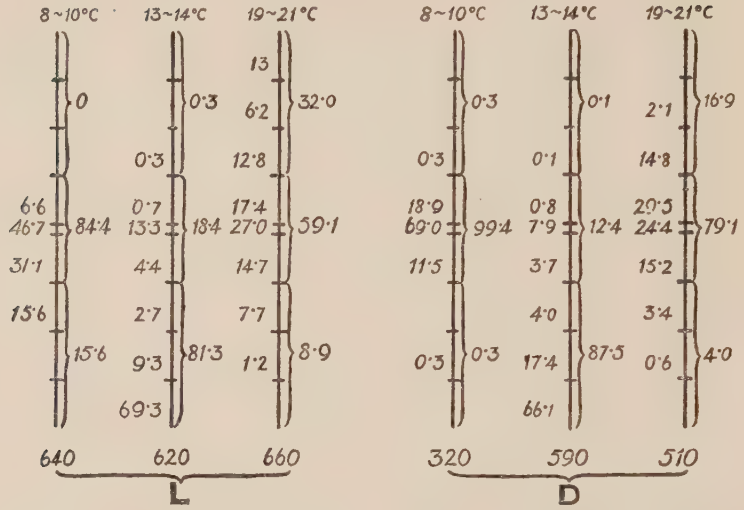

Diagram V.—Vertical migration of infective larvae of *Trichonema* in daylight (L) and in darkness (D), at different temperatures for 3 hours.

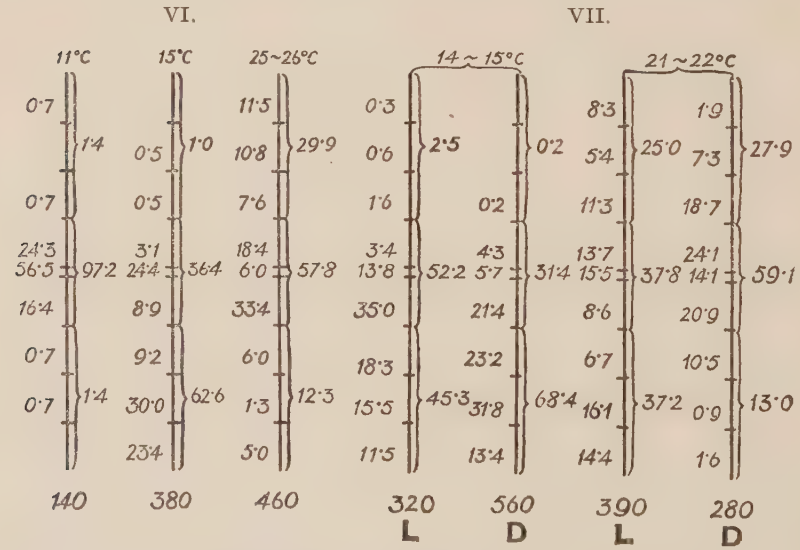

Diagram VI.—Vertical migration of infective larvae of *Haemonchus contortus* at different temperatures in daylight for 3 hours.

Diagram VII.—Vertical migration of infective larvae of *Haemonchus contortus* in daylight (L), from 12 to 3 p.m., and in darkness (D), for 3 hours, at two different temperatures.

geotropism. However, at 15°C. there is a strongly marked positive geotropism, and this is again seen in diagram VII, both in light and in darkness. At 21–22°C. the migration is as usual, indiscriminate. The relatively poor dispersal in darkness is apparent in this species as in *Trichonema*, and it is interesting to note that the positive geotropism at 14–15°C. is also rather more marked in darkness than in the light.

Vertical migrations of infective Hookworm larvae at different temperatures.—For comparison with the larvae of *Trichonema* and *H. contortus* which infect the host by being ingested with herbage, a few tests were carried

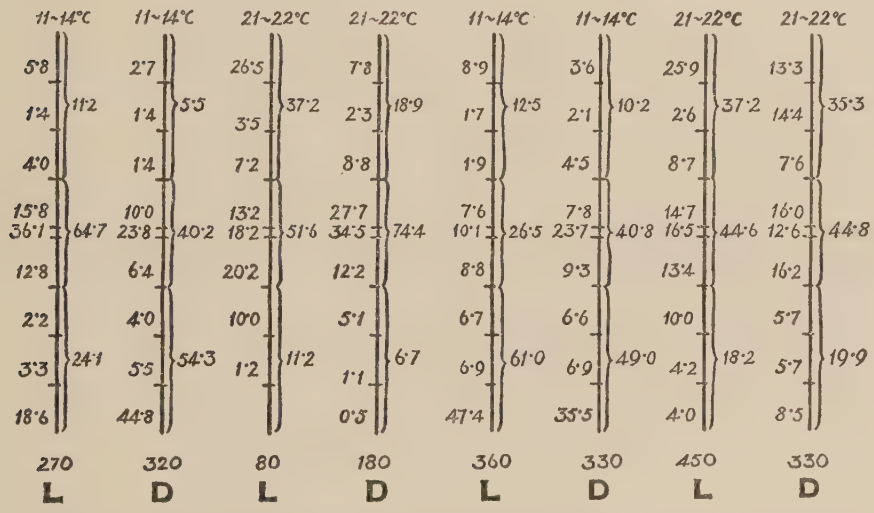

Diagram VIII.—Two similar tests with infective Hookworm larvae (*A. caninum*) showing their vertical migration at different temperatures in daylight (L) and in darkness (D).

out with the skin-penetrating larvae of *Ancylostoma caninum*. The results of two tests are seen in diagram VIII. At 11–14°C. there is evidently some degree of positive geotropism, which however is not nearly so well-marked as in the case of *Trichonema*, for quite a good proportion of the larvae have migrated on to the upper parts of the slide. At the higher temperature there is evidence of negative geotropism which is independent of the presence or absence of light. The duration of the tests was as usual 3 hours, from 12–3 p.m. and from 11 a.m. to 2 p.m., respectively.

Influence of light on lateral migrations of Trichonema and H. contortus larvae. Experiments to test the effect of light on the migrations of larvae on slides placed vertically gave inconclusive results. When the slides were placed horizontally the reactions of larvae to light were more positive and are illustrated in diagrams IX to XII. The slides in these tests were lying on a piece of plate glass which was adjusted absolutely levelly to avoid any gravity effects, and were at right angles to a window

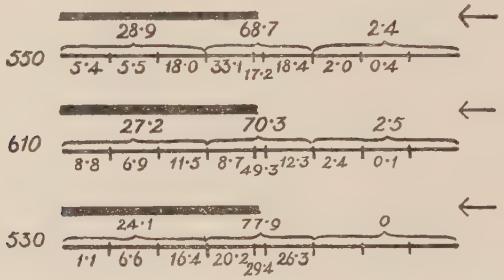

Diagram IX.—Illustrating the negative phototropism of infective larvae of *Trichonema* to daylight, 12 noon to 3 p.m.

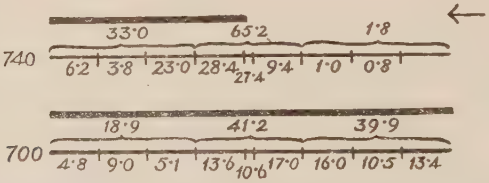

Diagram X.—Illustrating the negative phototropism of infective larvae of *Trichonema* to daylight, 11 a.m. to 2 p.m.

facing east. One half of each slide was covered with black paper about 5 mm. above it. The thick black lines in the diagrams represent the shaded part of the slide and the arrow indicates the direction of the source of light.

The three slides in diagram IX were exposed in this way simultaneously from 12 to 3 p.m., and in each of them the majority of the larvae are seen to have retreated from the light into the shade. In diagram X a similar result is seen in the first slide, while in the control slide, completely darkened, the migration is indiscriminate.

In diagram XI is shown the result of a simultaneous comparison of the effect of light on larvae of *H. contortus* and *Trichonema* from 12 to 3 p.m. The negative phototropism of *Trichonema* is here seen to be well marked while in the case of *H. contortus* it is barely evident. A similar series of slides on the same day from 3 to 6 p.m., is seen in diagram XII. The light

XI.

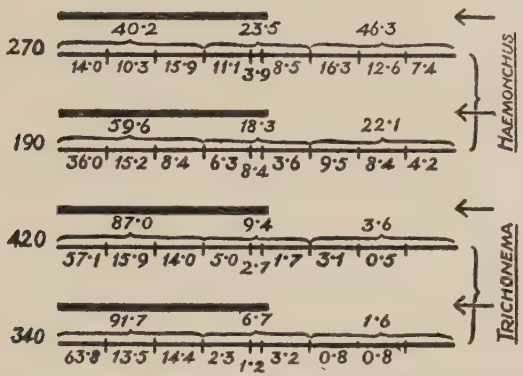

Diagram XI.—Simultaneous comparative test of the effect of daylight (12 noon to 3 p.m.) on the lateral migration of infective larvae of *H. contortus* and *Trichonema*.

XII.

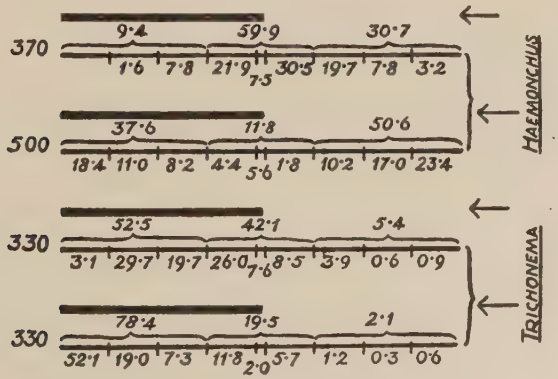

Diagram XII.—Same as in diagram XI (3 to 6 p.m.).

during this test was not so strong as in the previous one and in both pairs there has been relatively more migration into the illuminated part of the slide. It is evident from this, as has been shown by Rogers (1940), that the intensity of the light has an important influence on the migration of the larvae.

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Journal of Helminthology, Vol. XVIII, *No.* 4, *December*, 1940, *pp.* 183-192.

The Physiological Ageing of the Infective Larvae of *Haemonchus contortus*.

By W. P. Rogers, M.Sc., Ph.D.

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The method of estimating the numbers of infective Trichostrongyle larvae on pastures by recovering larvae from samples of herbage has become practical as the result of the work of Taylor (1939). A difficulty is apparent, however, owing to the fact that the larvae found on the grass would be of various ages and would vary in infectivity. It would be necessary, therefore, to select among the larvae collected from the herbage, those which would be capable of infecting animals before a true estimation of the danger of the pasture could be made. Again, as the collection of larvae by the Baermann technique depends on their activity and, therefore, on their physiological age, the accuracy of Taylor's method of estimating the efficiency of the Baermann larval recovery by adding a known number of larvae to one half of one of the herbage samples before extraction, relies on the similarity between the physiological ages of the larvae added and those originally on the grass. In view of the importance of Taylor's method of estimating pasture infestations, it seems necessary that the physiological ageing of Trichostrongyle larvae should be examined.

As yet, it appears that little work of this nature has been carried out with Trichostrongyle larvae. However, many workers have investigated the physiological ageing of Ancylostome larvae and the results should apply, to some extent, to Trichostrongyle larvae. Thus Payne (1922 and 1923), Cort (1925) and later Giovannola (1936) have shown that the physiological age of infective Ancylostome larvae can be determined by the examination of the fat stored in them. Rogers (1939) has determined the relationship between fat content, activity and infectivity in third stage hookworm larvae ageing under controlled conditions and the aim of the present work has been to find the similar relationships in Trichostrongyle larvae as exemplified by *Haemonchus contortus*.

PROCEDURE.

(*a*) *The Ageing of the Larvae.*—Larvae of *Haemonchus contortus* from a sheep were obtained by the usual faecal culture methods. The young infective forms were collected and about 20,000 were placed in each of three petri dishes in water approximately 2 mms. in depth. (It was necessary to store the larvae in large dishes in order to minimise the tendency of the larvae to form clumps which made subsequent counting very difficult.) The edges of the dishes were sealed with wax to prevent evaporation after which they were stored singly at 7°C., 24°C. and 37°C. At intervals, larvae were taken from the dishes and examined for activity, infectivity and fat content, following which the dishes were resealed and restored at the appropriate temperatures.

(*b*) *Infectivity Tests.*—Goats, the majority of which were one year old, were used to estimate the infectivity of the ageing larvae. Though considerable variation in infectivity due to the varying resistance of the animals used, was expected, only one goat could be spared for each test except that two goats were available for the test on young larvae. The animals used were free from Trichostrongyle infection, each showing only about 5 *Trichuris* eggs per gram of faeces. At the beginning of the experiments, two lots of 1,000 freshly cultured *H. contortus* infective larvae were counted and each lot was used to infect a goat. After four weeks, faecal examinations, by the sugar flotation of the eggs in one gram of faeces, was commenced. This was carried out each day for four weeks, after which the animals were killed and the number of *H. contortus* present determined. At intervals, lots of 1,000 larvae were counted from each of the cultures stored at 7°C., 24°C. and 37°C. and were used to infect goats. The subsequent egg output of the worms, and the number of worms present were found as before. In this way, the infectivity of the larvae which had been aged for known periods at known temperatures was found.

It was expected that at least 25% of the young larvae fed to the goats would prove infective but apparently these animals were resistant to *H. contortus* from sheep for the maximum infection obtained was only 113 worms. For this reason, all the resulting experimental infections were low.

(*c*) *Estimation of Fat Content.*—Before each infectivity test was carried out, larvae of the same physiological age were examined for fat content.

Exsheathment was stimulated by allowing the larvae to stand in 1/20 " Milton " solution in water for 2 hours after which they were fixed and stained with Sharlach R in the normal manner. It is considered that the treatment with " Milton " did not affect the fat content for after exsheathment the larvae were alive and active. The stained and mounted

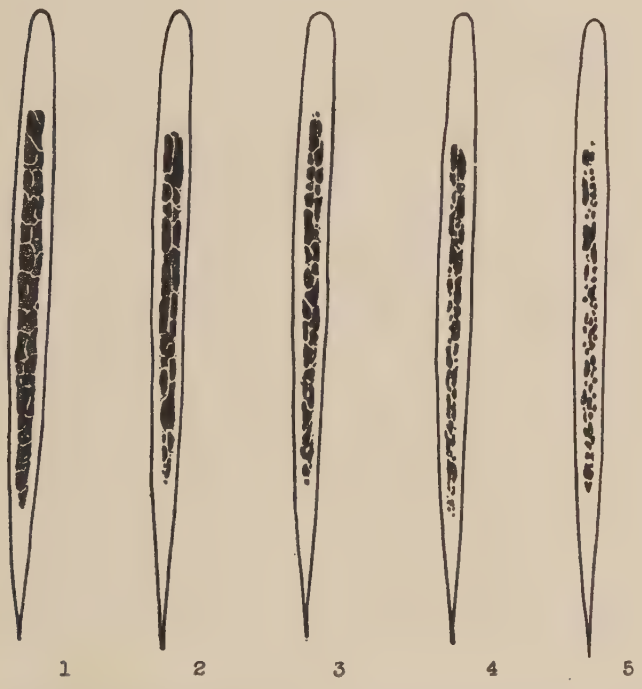


Fig. 1. Showing the fat globules found in larvae of different physiological ages. For explanation see Table I.

larvae were examined microscopically and the fat content estimated visually. As a rule, there was no great variation in the distribution and size of fat globules in larvae of the same physiological age.

(*d*) *Estimation of Activity*.—Larvae from the three sources (7°C., 24°C. and 37°C.) were taken and allowed to stand at room temperature

for one hour after which they were examined on a warm stage at 30°C. and the number of moves per minute made by 20 haphazardly selected larvae in each lot were counted. Before commencing counting, the larvae were allowed to remain on the warm stage for one minute. It was found that there was a great variation in the activity of the larvae of the same physiological age.

TABLE 1.

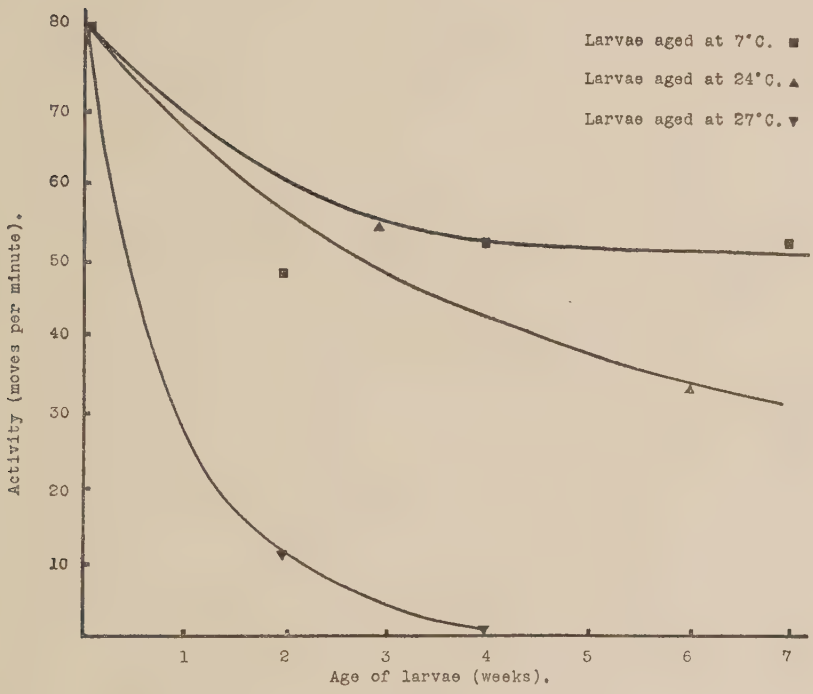
Age of larvae.	Storage temperature.	Fat content of the larvae.
Young larvae before storing		Many fat globules (all large) filling the cells along the line of the intestine. Oesophageal and tail regions free (see No. 1, Fig. 1). Diffuse stain throughout larvae, especially in the intestine.
2 weeks ...	7°C.	Many large globules and a few small ones along the line of the intestine (see No. 2, Fig. 1). Diffuse stain along the intestine.
4 weeks ...	7°C.	As for 2 weeks at 7°C. but the width of the fat cells along the intestine was slightly narrower (see No. 3, Fig. 2).
7 weeks ...	7°C.	Some large globules which were all elongated and narrow (especially in the anterior region) separated by smaller fat particles (see No. 4, Fig. 1). Some diffuse stain along the intestine. Width of the line of fat globules along the intestine was lessened.
2 weeks ...	37°C.	Small globules of fat only. Marked diffuse stain along the intestine.
4 weeks ...	37°C.	Diffuse stain along the intestine.
3 weeks ...	24°C.	Many globules of fat along the intestine, middle-sized and rounded in shape (see No. 5, Fig. 1). Diffuse stain throughout the body.
6 weeks ...	24°C.	Some small globules. Considerable diffuse stain throughout the body, especially along the intestine.

Table 1, giving the amounts of fat found in larvae stored for known periods at 7°C., 24°C. and 37°C.

OBSERVATIONS.

(*a*) *The Reduction in the Fat Content.*—Fig. 1 shows the amounts of fat found in the larvae of different physiological ages. It must be emphasised that the drawings are diagramatic representations only and

are included to indicate the amounts of fat in typical larvae of the various age groups. Though the variation in the fat content of the larvae of the same physiological age was not great, the appearance of the individual fat globules differed to some extent except in older larvae when they were



Graph 1. Showing the activity of larvae of various physiological ages.

usually uniformly round in shape. The diffuse fat staining seen in the larvae is not shown in Fig. 1, but is described in Table 1.

The fat in young larvae seemed to be evenly distributed throughout the cells of the mid-intestine and seldom seemed as concentrated as that found in young Ancylostome larvae (Rogers, 1939). As the larvae aged, the width of the band of fat in the intestine decreased and the individual globules of fat became more elongated and narrow. Later, the globules

became smaller and rounded in shape. In very old larvae little fat could be seen, a faint diffuse stain along the line of the intestine being the last evidence of its presence.

(*b*) *The Reduction in Activity.*—Graph 1 shows the activity of larvae of various physiological ages. It is evident that the larvae retained the ability to move in inverse relationship to the storage temperature. Thus larvae stored at 7°C. were active even after 7 weeks, whereas those at 37°C. were almost entirely without movement after 4 weeks. It appears that the rate of loss of the power of movement increases as the temperature rises for the larvae stored at 24°C., though less active than those at 7°C.,

Table 2.

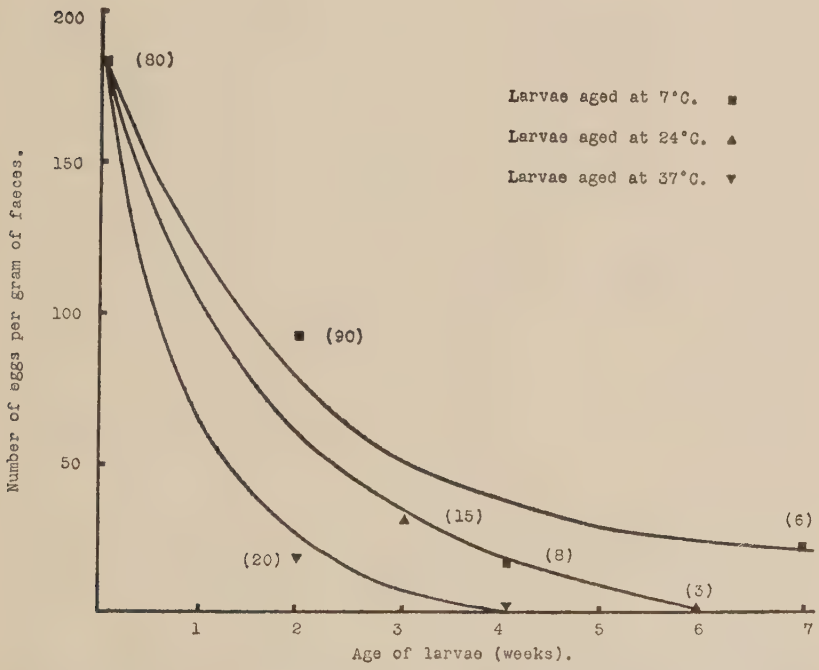
Larvae.		Average weekly egg count.				No. of worms.	
Age.	Storage temperature	1st week.	2nd week.	3rd week.	4th week.	♀♀	♂♂
Young ...	Before storing	53	65	149	234	72	41
Young ...	Before storing	23	74	97	142	33	14
2 weeks ...	7°C.	28	83	75	93	60	30
4 weeks ...	7°C.	8	10	24	16	7	1
7 weeks ...	7°C.	4	8	10	22	3	3
3 weeks ...	24°C.	5	7	27	33	9	6
6 weeks ...	24°C.	1	1	2	1	2	1
2 weeks ...	37°C.	10	28	25	17	10	10
4 weeks ...	37°C.	nil.	nil.	nil.	nil.	not examined	

Table 2, showing the weekly averages of the daily egg counts (sugar float, 1 grm. of faeces) in the infected goats. The first two entries show the results obtained from controls infected with larvae taken from fresh cultures.

were fairly active after 6 weeks, a period longer than that in which the 37°C. larvae were entirely exhausted.

(*c*) *The Reduction in Infectivity.*—Table 2 shows the infectivity of larvae of different physiological ages. Considerable error is probably introduced owing to the varying susceptibility of the experimental animals, but the figures certainly indicate the fall of infectivity as the larvae aged. Also, it appears possible to distinguish between the larvae aged at different temperatures, the infectivity falling most rapidly in those stored at higher temperatures,

The weekly averages of the egg counts carried out on the infected animals are also shown in Table 2. It is notable that there was some difference in the size of the infections in the two control animals. The egg counts correspond roughly with the number of worms recorded, the egg output per female varying from 1·5 to 7·1 eggs per gram of faeces. The fall in the egg counts as the physiological age of the infective larvae increased again demonstrates the steady loss in infectivity.



Graph 2. Showing the average daily egg counts (during the fourth week of infection) resulting from infections caused by larvae of different physiological ages. Figures shown between brackets give the number of worms found in each goat at post-mortem examination.

Graph 2, which shows the weekly average of the egg counts in the different experimental animals during the fourth week of infection, clearly indicates the fall in infectivity as the larvae were aged. The figures shown between brackets give the total number of worms found in each goat on post-mortem examination.

(*d*) *The Relationship between Fat Content, Activity and Infectivity.*— The relationship between fat content and activity seemed somewhat closer than between either of these and infectivity, especially in slowly aged larvae. Thus the fat content and activity in the larvae kept at 7°C. remained high, both being reduced by about ⅓ after 7 weeks of storage, whereas infections fell from 80 worms to 6. At 24°C. the relationship between the three factors was closer and at 37°C., fat content, activity and infectivity fell almost at similar rates. It may be noted here that the larvae stored at 37°C. exsheathed in large numbers during storage and this probably caused them to age more quickly, or at least increased the rate of loss of infectivity.

It appears that *H. contortus* 3rd. stage larvae age more quickly than infective Ancylostome larvae for it was found that 20% of the latter forms were " infective " (Goodey's (1922 and 1925) " floating raft " method was used for estimating infectivity) after 8 weeks storage at 7°C. However, the tests for infectivity on Trichostrongle larvae were carried out on animals which apparently had a high natural resistance to *H. contortus* from the sheep and the infectivity may have been much higher if the normal hosts had been used as the experimental animals.

Discussion.

The difficulty of estimating the infectivity of Trichostrongle larvae is increased by the fact that the relationship between the infectivity, fat content and activity depends somewhat on the rate of ageing. However, bearing in mind that after periods of cold weather the infectivity would be somewhat lower than indicated by the fat content and activity and vice versa in hot weather, indications may be given for the rough estimation of infectivity by the examination of the other factors concerned. Thus forms in which the column of fat along the intestine is well marked and broad and which have an activity of over 70 moves per minute at 30°C. would be considered to be highly infective. When the fat globules have become smaller, separated and rounded and the activity is less than 40 moves per second, the infectivity would probably be low. Intermediate forms would have a line of elongated fat globules along the line of the intestine.

In practice, the larvae recovered from herbage would be of various ages and it would be extremely difficult even to form a rough estimate of the average infectivity. Furthermore, the infectivity to sheep would probably be the matter for determination under normal circumstances and the present experiments deal with goats only.

Summary.

1. At known periods the infectivity, fat content and activity of larvae of *Haemonchus contortus* aged at 7°C., 24°C. and 37°C., were examined.

2. The relationship between fat content, activity and infectivity was found to be fairly close in rapidly aged larvae. In slowly aged larvae the infectivity fell more rapidly than the other factors.

3. It was found that forms in which the line of fat along the intestine was well marked and broad and the activity was over 70 moves per minute at 30°C. may be regarded as comparatively highly infective. Larvae in which the fat globules were smaller, separated and round and had an activity of less than 40 moves per minute were found to have a low infectivity.

Acknowledgments.

The author is greatly indebted to Mr. J. W. G. Leiper, M.R.C.V.S., who prepared the larvae for the infection of the experimental animals and carried out a large number of egg counts.

This work was carried during the tenure of a Hackett Studentship of the University of Western Australia.

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Journal of Helminthology, Vol. XVIII, *No. 4, December,* 1940, *pp.* 193-208.

On the Specific Status of the so-called Biological Strains of *Heterodera schachtii* Schmidt.

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The genus *Heterodera* comprises three species, the root-knot nematode, *H. marioni* (Cornu 1879) Goodey 1932, and the two species in which cysts are produced. These are, *H. schachtii* Schmidt 1871, which is associated with disease in potatoes, sugar beet, cereals, peas and some other crops, and *H. punctata* Thorne 1928, which has caused disease of wheat in Canada, and has been found in this country infesting a common grass *Agrostis stolonifera* L.

Included in the species *H. schachtii* are at least four biologically distinct races. They have well-defined and fairly limited host-ranges, and certain slight differences in morphology. The morphological differences so far noted have been in larval length and in the shape and surface markings of the cyst formed from the dead female. It has been shown that the larvae of certain populations of the oat strain, for example, are significantly longer than those of certain populations of the beet and potato strains. Sufficient evidence is not yet available to state whether this holds good for all populations of these strains, but it seems probable that the oat strain larvae are always longer than those of all other known strains. Less pronounced differences which have been shown to occur between the larvae of some other strains may or may not be constant for all populations of these strains (Franklin 1940). When the cysts of the various strains are compared, those of the potato strain are seen to differ considerably from the others: they are approximately spherical, and have minute punctate markings arranged in fairly definite rows, while in all the other strains there is a knob bearing the vulva on the posterior end of the cyst, giving it a lemon-shaped appearance, and the punctations are irregularly arranged. There are also slight differences in the shade of brown of the dry cysts of the various strains, which can be distinguished

when a number of cysts is seen together. The size and proportions of the cysts of the different strains also vary somewhat, but these characters vary a good deal within the strains, so that it is practically impossible to determine the strain of an isolated lemon-shaped cyst, unless it contains the large larvae which are characteristic of the oat strain.

Hitherto no differences have been recorded between the males of the various strains, except in the degree of development of the lateral wing in the caudal region (Triffitt 1928). In view of the distinctiveness of the cysts of the potato strain, it was considered possible that the males also of this strain might differ from those of some of the other strains. An examination was therefore made of males of five strains of *Heterodera*.

Methods.

The most satisfactory source from which to obtain males in large numbers was the infected roots of the various host plants. Roots were chosen which bore numerous white females just bursting through the cortex. The roots were washed free from soil, put in a covered dish and kept moist for several days. At intervals of two or three days the roots and dish were washed out with water, the water was allowed to stand until the nematodes had sunk to the bottom of the vessel, and the male *Heterodera* were picked out on a needle. Males were obtained in this way over a period of ten days or a fortnight from one sample of roots. The worms were killed in a drop of water by gentle heat, and preserved in 5% formalin. In most cases, when the worms are killed by heat, the spicules are protruded and can be examined without difficulty.

For purposes of measurement, outline drawings were made of numerous specimens under the camera lucida at a magnification of about 120 diameters. The more detailed examinations were carried out under an oil-immersion lens giving a magnification of 2640 diameters.

Observations.

When examining males at a high magnification, special attention was paid to the details of the head region, particularly to the buccal skeleton and the mouth stylet, to the spicules and the length of the tail.

No striking morphological differences were found in the head region, apart from slight differences in the length of the stylet. Slight variations were observed in the length of the tail and in the degree to which the cuticular striations could be seen, but these were not constant.

Definite differences were however observed between the spicules in the various strains. The size and general shape of the spicules are similar in the different strains, but the distal pointed end varies. When observed in lateral view, one type of spicule ends in a single tooth, another type has two teeth and a third has three teeth. In all cases the two spicules of the pair are alike, and the type of spicule in each strain is constant. The differences can only be seen under the highest power of the microscope.

Morphology of the Spicules.

1. *Pea Strain.*

This was the first strain in which the distinctive form of the spicule tip was observed. It was noticed that the distal ends of the spicules seemed to be truncate and jagged. On closer examination of the tips of the spicules of several specimens it was found that, seen laterally, they terminated in three distinct, equal, sharply-pointed teeth (Fig. 4). This type of spicule tip was seen in males of the pea strain from peas and from broad beans, but not in any of the other strains examined.

2. *Beet Strain.*

The spicules of males of the beet strain, when seen from the side, were found to end in two equal well-defined teeth (Fig. 3). Many males from infected mangolds and radishes were examined, and all showed this character. In one or two favourable cases a very delicate transparent wing was seen running along the edge of the distal part of the spicule when viewed dorsally.

3. *Strain from Curled Dock and Hops.*

Amongst some preserved material of curled dock, *Rumex crispus*, infected with *H. schachtii*, a single male was found. In this the spicule tips were bifid, as in the beet strain.

Another male *Heterodera* was found with some preserved infected hop roots and this also had the bifid type of spicule. It seems probable, therefore, that the males of these two strains resemble those of the beet strain, though there is a somewhat remote possibility that the specimens did not come from the host plant with which they were found.

4. *Potato Strain.*

The spicules of the males of this strain end in a single point, but this appears to be of a different material from that of which the main body of the spicule is composed (Fig. 2). It seems to be thinner than the rest

of the spicule, and of a similar substance to that which forms a delicate, highly transparent, wing-like flange which runs down the side of the distal limb of the spicule. This flange can be seen when the spicule is viewed from the dorsal aspect, and resembles a similar structure which was occasionally seen in spicules of the beet strain males (Fig. 1).

5. *Oat Strain.*

Owing to the lateness in the season it was impossible to get live males of the oat strain from living plants, but a few specimens were found amongst some preserved material of oat roots infected with *H. schachtii*. This material was very kindly given me by Dr. T. Goodey, who had received it from Germany. The spicules of these males are rather more slender than those of the potato strain, but resemble them in not being toothed at the distal end. The whole spicule appears to be twisted and to have the edges rolled, so that the narrow distal end is tubular. What appears to be the opening into this tubular portion can be seen in ventral view (Fig. 9).

6. *Sea Maram Grass Strain.*

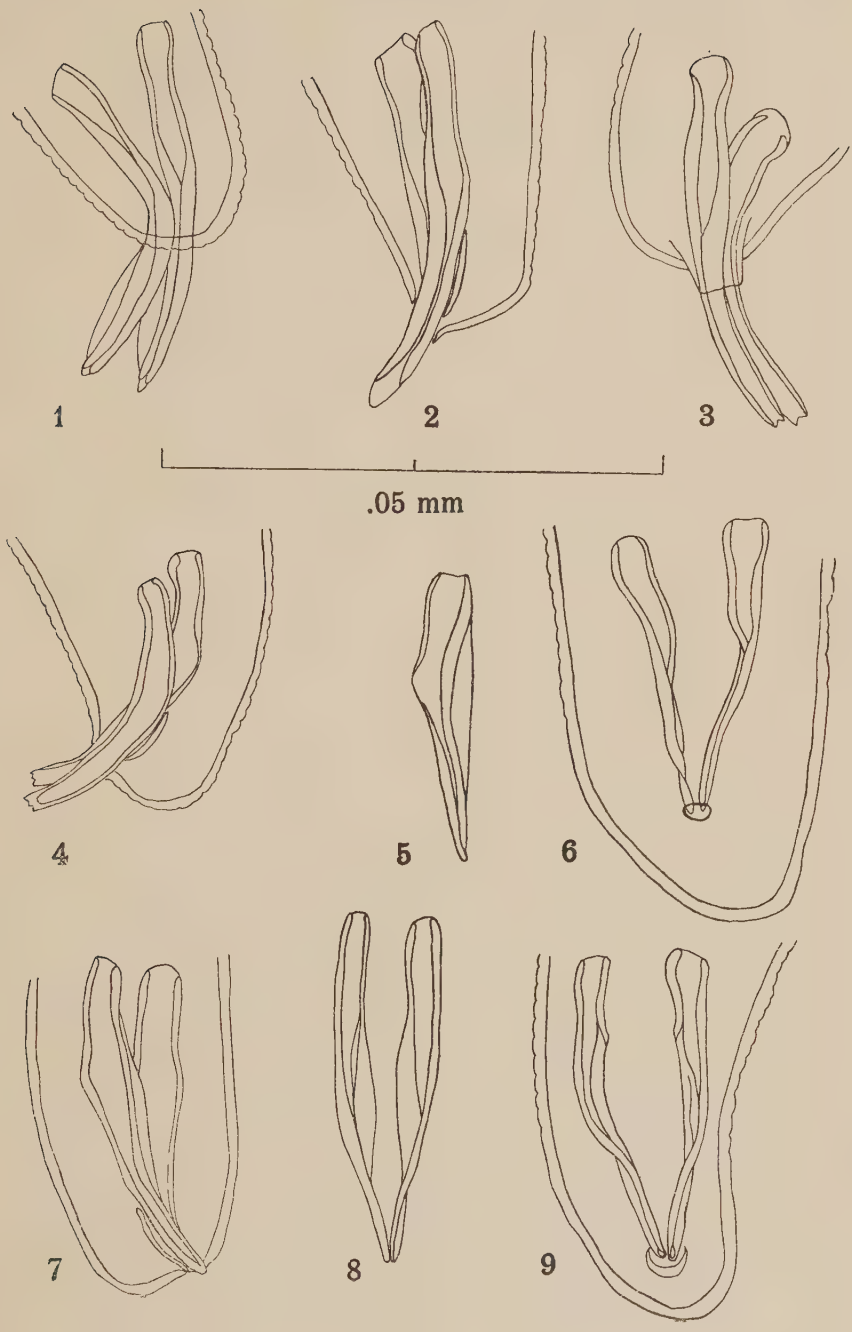
A few males from the gelatinous material attached to the posterior part of the females on the roots of some infected preserved sea maram grass (*Ammophila arundinacea* Host.) had spicules rather similar to those from the oat material, but no opening into a tube formed by the rolling of the distal limb could be seen at the tip of the spicule (Figs. 5 & 6).

7. *Heterodera punctata.*

Dr. T. Goodey very kindly lent me a slide on which were mounted three male *Heterodera* which he had obtained from turf. They are probably males of the species *H. punctata*, which has been found parasitising the grass *Agrostis stolonifera* L. growing in the locality from which the turf was taken. The spicules of these worms end in simple points, but the distal part of the spicule does not appear to be twisted and rolled to the same extent as it is in the spicules of the oat and maram grass strains (Figs. 7 & 8).

Heterodera spicules.

Fig. 1. From potato—dorsal. Fig. 2. From potato—lateral, showing gubernaculum.
Fig. 3. ,, mangold—lateral. Fig. 4. From pea—lateral, showing gubernaculum.
Fig. 5. ,, sea maram grass—isolated spicule, lateral.
Fig. 6. ,, ,, ,, ,, —ventral.
Fig. 7. Probably *H. punctata*—lateral, showing gubernaculum.
Fig. 8. ,, ,, ,, —dorsal. Fig. 9. From oat—ventral.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8. *Heterodera marioni.*

A slide with a single male of the species *H. marioni* was also kindly lent to me by Dr. Goodey. It was not very favourable for examination of the spicules, but they appeared to be not unlike those of the strain of *Heterodera* from sea maram grass.

Measurements of Males.

1. *Body Length.*

Large numbers of outline drawings of males of three strains of *Heterodera* were made as described above, in order to compare their mean lengths. The following results were obtained :—

Strain.	Host.	Locality	No. measured	Mean mm.	Standard error.
Beet ...	Mangolds	Herts.	100	1·468	0·0095
,, ...	Radishes	,,	11	1·463	0·0375
Pea ...	Peas	,,	53	1·295	0·0188
,, ...	Broad beans	Glos.	25	1·206	0·0303
Potato ...	Potatoes	Herts.	100	1·113	0·0089

These measurements were compared statistically, and it was found that the mean lengths of the males from mangold and radish do not differ significantly, but that there are significant differences between the mean lengths of males of all the other strains. These differences are probably of no great importance, as it will be seen that males from peas and from broad beans (which plants are attacked by the same strain of nematodes) differ considerably in length. The difference may be due to a host reaction, or to the fact that the worms measured came from two different populations. The mangold and radish material, though from different hosts, came from the same plot of land. Triffitt (1929a) found considerable variations in the length and proportions of males of the beet strain from a number of different host plants.

2. *Stylet Length.*

The mouth stylets of ten males each of the beet, pea and potato strains were measured. It was found that the mean length of the stylet in the beet strain was significantly greater than that of both pea and potato

strains, but that the difference between the pea and potato strains was insignificant.

Mean stylet length in beet strain	29·71 ± 0·4104μ
,, ,, ,, pea ,, ...	27·54 ± 0·4047μ
,, ,, ,, potato ,, ...	27·39 ± 0·4773μ
Difference between means of beet and pea strains	2·17 ± 0·5763μ
,, ,, ,, beet and potato ,,	2·32 ± 0·6295μ
,, ,, ,, pea and potato ,,	0·15 ± 0·6257μ

3. *Spicule Length.*

In order to measure the length of the spicules, outline drawings of them were made and these were measured. Accurate measurements are difficult, as the spicules are not flat in any plane. The curvature in the dorso-ventral plane is considerable, but it is much less in the lateral plane, and as far as possible measurements were therefore made from the lateral aspect. Ten spicules were measured in each of the three strains, only one of the pair being considered in each individual. No significant differences were found between the mean lengths of the spicules of the three strains.

Mean length of spicule in beet strain ...	34·625 ± 1·052μ
,, ,, ,, potato ,, ...	34·293 ± 0·803μ
,, ,, ,, pea ,, ...	32·514 ± 0·163μ

Length/Breadth Ratios of Cysts.

In view of the distinct differences found between the males of the several strains of *H. schachtii*, a further effort was made to discover whether there may not be measurable differences between the cysts of these strains, apart from those differences already known. It has been shown that the rounded cysts of the potato strain have different surface markings from those of the lemon-shaped strains, but no differences have yet been observed between the cysts of the many lemon-shaped forms, except in the shade of brown of the dry cyst (a character which it is impossible to define accurately), and in the ratio of length to breadth in two of the strains. This latter character was measured by Goffart (1930 & 1932), who found that the length/breadth ratio of beet strain cysts differed from that of oat strain cysts. It is obvious from the figures given by different workers that there is considerable variation in the size of the cysts of any one strain. One might expect to find some variability in size between different populations of any given strain. This no doubt depends on a number of factors, such as the species and health of the host plant, the

time taken for the cyst to develop, and the environment during its growth. The general shape of the cysts of a given strain appears to be a more constant feature than the dimensions. For example, pea strain cysts of all sizes appear to be more spherical than those of the oat strain. In order to find out whether this difference in shape is real, and whether such differences occur between many of the strains, the length/breadth ratio was calculated for a large number of cysts. For length, the longitudinal axis excluding the neck was measured, as it was thought that there would be a danger of obtaining false measurements if the neck were included, owing to the possibility of its having been broken off short. Its length may also depend somewhat on the thickness of the host root, since in a thick root, in order for the cyst to protrude while the head remains embedded in the endodermis of the stele, the neck must be longer than if the development is taking place in a fine rootlet. It was not always easy to judge exactly where the neck and body met in a cyst, but in doubtful cases a point was taken which would lie on a line continuing the curve of the general outline of the cyst across the neck region. When a cyst was malformed, with the neck placed almost laterally, the position of the neck was ignored, and a measurement made of the longitudinal axis of the body, terminating at the vulva.

One hundred cysts from 12 different samples have been measured. Eight strains are included, four of them being represented by two samples of cysts each: the beet strain is represented by cysts from mangolds and radishes grown in the same soil, the pea strain by cysts from peas and broad beans grown in different localities, the potato strain by cysts from potatoes and tomatoes grown in different soil, and the clover strain by cysts from *Trifolium repens* grown in Kent and in Hertfordshire.

The mean length/breadth ratios for the 12 samples of cysts are given in Table I.

These values have been compared statistically by the method of analysis of variance. The ratio of variance between the strains to error variance was found to be highly significant, and the mean values may therefore be compared. The standard deviation for the 1,200 values was 0·1591 and the critical difference between any two means was calculated to be 0·0441 at the 0·05 level of probability. Differences greater than this are considered to be significant, that is they would occur by chance fewer than 5 times in 100 samples of the size taken, and their occurrence is probably therefore due to some factor other than chance.

A comparison of the differences between the means shows that cysts within each of the following three groups do not differ significantly from one another in shape, as measured by the ratio of length to breadth.

1. Kent clover, radish, Herts clover and mangold strains and *Heterodera punctata*.
2. *H. punctata* and Herts clover, mangold and oat strains.
3. *Myosotis* and broad bean strains.

TABLE I

Host.	Mean.	Difference.	Variation Coefficient.
Clover (Kent)	1·5164	0·0222	10·84%
Radish	1·4942	0·0155	12·78%
Agrostis (*H. punctata*) ...	1·4787	0·0013	10·51%
Clover (Herts.)	1·4774	0·0011	12·00%
Mangold	1·4763	0·0377	12·27%
Oats	1·4386	0·0925	10·97%
*Grasses	1·3461	0·0495	11·32%
Myosotis	1·2966	0·0205	10·38%
Broad bean	1·2761	0·0599	13·06%
Pea	1·2162	0·0697	10·85%
Tomato	1·1465	0·0741	15·43%
Potato	1·0724		8·86%

Critical difference 0·0441

*(The cysts of this strain were lemon-shaped and came from soil from a Leeds bowling green where the grasses were parasitised.)

With the exception of the *Myosotis* and broad bean strains, the last six in the list are all significantly different in shape from one another.

It is evident from these observations that there may be real differences in shape between the cysts of some strains. That the differences in shape are not necessarily due to the locality or to the host species is shown by the similarity of the ratios of the two clover strains and of the radish and mangold strains respectively. In the case of the pea and potato strains,

however, there are significant differences between cysts of the same strain from different hosts.

Goffart (1930 & 1932) also found significant differences between the length/breadth ratios of cysts of the beet, oat and potato strains, but no differences between cysts of the beet strains from several host species, and from different localities. His values are slightly lower than those here recorded, but are similarly related to one another. He gives the ratio of " trunk length " to breadth for beet strain cysts as 1·4, for oat strain cysts as 1·25 and 1·39 (two populations), and for potato strain cysts as 1·038.

It seems to be doubtful whether, even if large numbers of measurements were made, the value of the mean length/breadth ratio of a sample of cysts would ever be a reliable means of determining the strain.

Systematics.

The characteristics of *Heterodera punctata* and of the four better-known strains of *H. schachtii* may conveniently be summarised and contrasted with the aid of Table II.

Until now, the so-called strains of *Heterodera schachtii* have been considered as biological races of a single species, of which different populations had become adapted to certain agricultural crops. Some, indeed, had become so highly specialised that they appeared to be incapable of attacking any but a very few plant species. The differences in the morphology of the spicules, and in the surface markings of the cyst wall, however, appear to be more fundamental than any which might arise from a mere change of host, or from an adaptation to one or two host species. These morphological differences fall into line with biological differences which have been noted by those who have been concerned with *Heterodera* both in the laboratory and in the field.

These differences between the four strains of *H. schachtii* seem to the writer to demand the recognition of four species. Four species are therefore designated as follows :—

1. *Heterodera schachtii* Schmidt 1871.

Synonym *Heterodera schachtii* A. Schmidt subsp. *minor* O. Schmidt 1930.

The sugar beet nematode, first found by Schacht in 1859, on the roots of sugar beet, and named *Heterodera schachtii* by A. Schmidt in 1871, was renamed *H. schachtii* subsp. *minor* by O. Schmidt in 1930 to distinguish

TABLE II.

	Beet strain	Potato strain	Pea strain	Oat strain	*H. punctata*
Cysts—					
Shape	Lemon	Spherical	Lemon	Lemon	Ovoid
Vulva	Prominent	Not prominent	Prominent	Prominent	Not prominent
Anus	Combined with vulva (as Fig. 12)	Distinct, minute (Fig. 10)	Combined with vulva	Combined with vulva	Distinct, same size as vulva (Fig. 11)
Length/breadth ...	1·4–1·5 (Goffart 1·3–1·4)	1·06–1·09 (Goffart 1·038)	1·2–1·3	1·4–1·5 (Goffart 1·2–1·4)	1·4–1·5
Punctations	Irregular	In rows	Irregular	Irregular	In rows
" Sub-crystalline layer "	Present	Absent	Present	Present	Present
Protection of eggs in cyst	Effective	Effective	Effective	Effective	Ineffective against drying
Larvae					
Length	400–500μ	400–500μ	400–500μ	550–600μ	550–600μ
Tail	Pointed	Pointed	Pointed	Pointed	Acutely pointed
Males					
Length	1·4–1·5 mm.	1·1–1·2 mm.	1·1–1·3 mm.	1·2–1·5 mm.	0·9–1·3 mm. (Thorne)
Stylet	29–30μ	27–28μ	27–28μ	28μ (Triffitt)	—
Spicules	34–36μ	34–35μ	32–33μ	36μ ,,	—
Spicule tip	Bifid	Single, transparent	Truncate, tridentate	Single, limb rolled	Probably single
Bionomics					
Hatching in response to root excretions	Almost at any time of year	Almost at any time of year	Only at certain periods	None observed	—
Minimum period between embryonation and hatching	A few weeks	A few weeks	—	9 months (Goffart)	—
Known host range ...	*Chenopodiaceae* & *Cruciferae*	A few *Solanaceae*	Peas, beans and vetches	Cereals	Wheat and *Agrostis stolonifera*

Comparison of *H. punctata* and four " strains " of *H. schachtii*.

it from the oat nematode which has much longer larvae. Now that the so-called " beet strain " of this nematode becomes a species, it regains its original name, as it was first described and named *H. schachtii* when found parasitising sugar beet.

H. schachtii is characterised by its lemon-shaped cysts bearing a white " sub-crystalline " layer when newly formed, and having irregularly arranged punctate markings on the wall. The cyst is rather elongated, with a length/breadth ratio of 1·3–1·5. The wall is sufficiently thick to prevent the cyst contents from drying. The larvae are small as compared with those of some of the other *Heterodera* species, being 400–500μ in length. The males have comparatively long stylets of 29–30μ, and bifid-tipped spicules 34–36μ long. The larvae are stimulated to hatch in the presence of root excretions of the host plant throughout the year, and are capable of hatching in the same season in which they develope. The host range is fairly wide, many members of the *Chenopodiaceae* and *Cruciferae* being attacked, and possibly a number of other plants.

2. *Heterodera rostochiensis* Wollenweber 1923.

Synonyms *Heterodera schachtii* forma *solani* Zimmermann 1927.

Heterodera schachtii subsp. *rostochiensis* (Woll.) Kemner 1929.

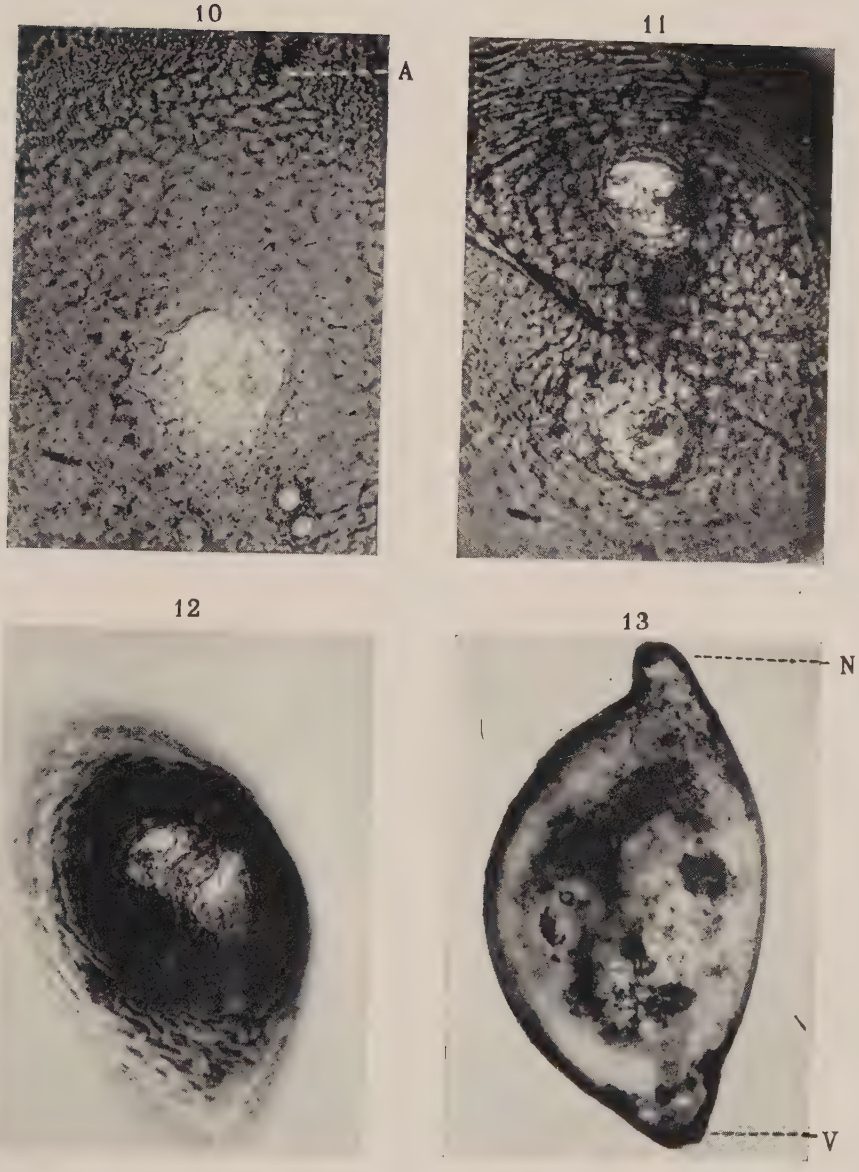
The " potato strain " of *H. schachtii* had been known for some years before Wollenweber recognised that this eelworm formed cysts which differed in shape from those formed by *H. schachtii* on sugar beet. He named the potato eelworm *Heterodera rostochiensis*, as the infection which he investigated was at Rostock. It has since been claimed by Zimmermann (1927) and by Goffart (1928) that the potato eelworm can be made to attack sugar beet, and that on this host it produces lemon-shaped cysts, but other workers have been unable to confirm this, or to bring about the transference of beet nematodes to potatoes. There is therefore no valid evidence that the beet and potato eelworms are one and the same, and Wollenweber's name, *Heterodera rostochiensis* is adopted for the potato eelworm.

Fig. 10. Posterior end of cyst of *H. rostochiensis* showing large vulva and minute anus (A). The punctations on the cyst wall can be seen near the top of the photograph, but the regular arrangement cannot be distinguished. The two circular patches just below the vulva are foreign matter. (×500)

Fig. 11. Posterior end of cyst of *H. punctata* showing anus and vulva of equal size. On the lower left-hand side can be seen rows of punctations. (×500)

Fig. 12. Posterior end of a lemon-shaped cyst showing anus and vulva close together. (×500)

Fig. 13. Typical lemon-shaped cyst. N=neck, V=vulva. (×100)



Diagnostic features of *Heterodera* cysts.

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H. rostochiensis is characterised by having rounded cysts with the vulva on a level with the cyst wall. The anus is minute and difficult to see, but the vulva can quite easily be seen at the posterior end of the cyst (Fig. 10). The cyst wall is marked with horizontal rows of punctations and has no well defined " sub-crystalline " layer, even on the newly formed cyst. The larvae are comparatively small, 400–500μ in length, and are well protected from drying while inside the cyst. The males have a stylet of 27–28μ, and spicules of 34–35μ ending in a single point composed of a more delicate material than that of which the limb of the spicule consists. The larvae can be stimulated to hatch by the presence of host root excretions during the greater part of the year, and are capable of hatching within a few weeks of development. The host range is limited to a few members of the *Solanaceae*.

3. *Heterodera göttingiana* Liebscher 1890.

Liebscher recorded an attack of *Heterodera* on peas in Germany in 1890. He considered that the pea nematode differed sufficiently from the strain known on sugar beet to be considered a separate species, and he named it *Heterodera göttingiana*, as it occurred at Göttingen. This name has been revived for the eelworm found attacking peas in Britain, as it seems likely that it is the same as that described by Liebscher.

Heterodera göttingiana has lemon-shaped cysts with a rather low ratio of length to breadth, namely 1·2–1·3 in the populations measured. The cyst wall bears irregularly arranged punctate markings, and there is a " sub-crystalline " layer on the new cyst. The larvae are of the small type, 400–500μ long, and are adequately protected from drying while inside the cyst. The males have a stylet of 27–28μ in length and the spicules are 32–33μ long. They are truncate at the tip and have three sharp teeth of approximately equal size. The larvae, according to the writer's observations, are stimulated to hatch from the cysts by the presence of pea root excretion in the summer, but appear to be dormant in early spring. The plants known to be attacked by this species are peas, beans and vetches.

4. *Heterodera major* (O. Schmidt 1930).

Synonym *Heterodera schachtii* A. Schmidt subsp. *major* O. Schmidt 1930.

Kühn in 1874 was the first to record this eelworm as a parasite of cereals, and he considered that it was identical with the sugar beet nematode

Heterodera schachtii. Otto Schmidt in 1930 and 1931, and Goffart in 1932, recognised that the "oat strain" of *Heterodera* differed both in bionomics and in the size of the larvae from the "beet strain." The larvae were shown to be considerably longer than those of the beet nematode, and Schmidt therefore named the oat eelworm *Heterodera schachtii* subsp. *major*. That the strain which attacks oats in England is the same as that investigated by the German workers seems probable owing to the similarity in the length of the larvae, and to the difference in length between these larvae and those of the eelworms parasitic on sugar beet, peas and potatoes in both countries. Now that the male has been found to have spicules different from those in the beet, pea and potato nematodes, the oat eelworm may be regarded as a separate species. By article 12 of the International Rules of Zoological Nomenclature, which requires that "A specific name becomes a subspecific name when the species so named becomes a subspecies, and *vice versa*", this species must be given the name *Heterodera major*. It is perhaps an unfortunate name, as it refers to the larval stage of the nematode, while the adults do not differ greatly in size from those of other species of the genus. Also, the larvae are of the same order of magnitude as those of *H. punctata*.

Heterodera major has lemon-shaped cysts, which, in the English strains measured, have a length/breadth ratio of 1·4–1·5, but were rather more rounded in Goffart's strains (1·2–1·4). The cyst wall is marked with irregularly arranged punctations, has a well defined "sub-crystalline" layer, and protects the contents from drying. The larvae are large, 550–600 μ long. According to Triffitt the mouth stylet in the male is 28 μ in length and the spicules are 36 μ long. The spicules have a single point and the distal limb appears to be rolled into a tube. Stimulation of the larvae to hatch has not been found to occur to any extent at any time of the year in the presence of oat root excretion under laboratory conditions. According to Goffart (1939) the larvae require nine months to mature, that is to say, nine months must elapse after the cyst has been formed before the larvae are capable of hatching. Cereals are the only known hosts of this species.

Conclusions.

Since the differences between the various races of *Heterodera* have been shown to be fundamental, it seems reasonable to assume that each species will probably confine its attacks fairly constantly to its own host range.

When it was thought that the various races had originated from a single unspecialised strain at no very distant date, it was always considered possible that a reversion might occur and that any strain, exceptionally, might be capable of returning to its supposed original unspecialised habits. Though the host-ranges of two or more species of *Heterodera* may overlap, as is the case with *H. punctata* and *H. major*, both of which parasitise cereals, it would appear unlikely that a species would, in the course of two or three years, become adapted to the normal host of another species. Thus there is less risk of, for example, " oat sick " land being dangerous to mangolds than one would expect if a biological strain only were involved.

There remain several strains of *Heterodera* which cannot yet be assigned to any of these species, as their morphology is incompletely known. Hops, clover and sea maram grass are well known hosts in this country, and a number of others have been recorded in other parts of the world. Corder, Buhrer and Thorne (1936) list 143 hosts of the cyst-forming species of *Heterodera*, many of the records no doubt not being due to the sugar beet nematode as now defined. It appears possible, however, that *H. schachtii* is the least specialised species of *Heterodera*, and may be responsible for attacking a wide variety of plants.

It is possible that, amongst the incompletely known races of *Heterodera*, such as that described by Triffitt (1929b) from sea maram grass (*Ammophila arundinacea*), forms will be found which differ from any of the species so far known, and other species will have to be made. The sea maram grass form, indeed, differs from any other known form, as Triffitt points out, in that males are always found in the jelly-like substance which is attached to the posterior end of the mature cyst, and in which embryonated eggs are also usually found. The spicules in the male of this race are as yet insufficiently well known for an accurate description of their morphology to be given.

Now that the differences between the spicules have been discovered, it is to be hoped that, when new hosts of *Heterodera* are found, it will be possible to identify the species with certainty.

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